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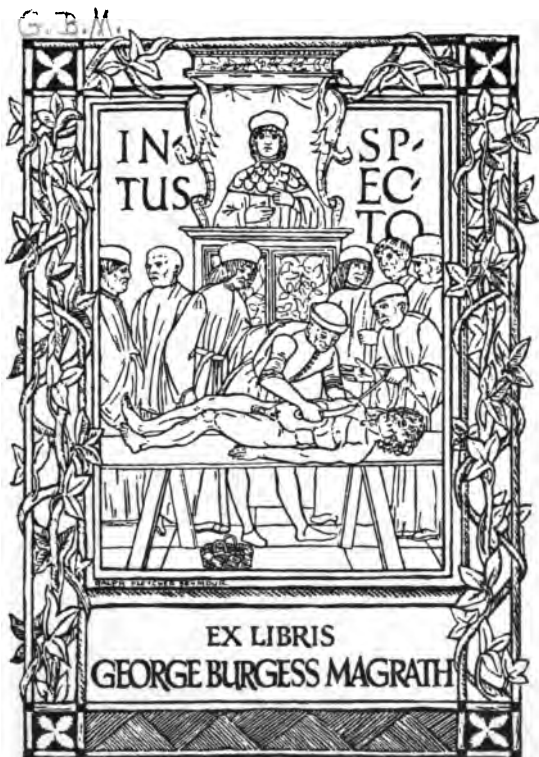
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**BACTERIOLOGICAL DIAGNOSIS**

**FOR**

**PRACTITIONERS**

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HANDBOOK  
OF  
BACTERIOLOGICAL DIAGNOSIS  
FOR  
PRACTITIONERS

*INCLUDING INSTRUCTIONS FOR THE CLINICAL  
EXAMINATION OF THE BLOOD*

BY

*Walter* D'ESTE EMERY, M.D., B.Sc. LOND.

LECTURER ON PATHOLOGY AND BACTERIOLOGY IN THE UNIVERSITY OF  
BIRMINGHAM

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## PREFACE.

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No practitioner who wishes to do his best for his patients and to promote his own interests can afford to neglect any means of clinical investigation which may help him to arrive at a correct diagnosis, and offer hints as to prognosis and treatment. Pre-eminent among the more recent methods of investigation are those which are applied by the bacteriologist; and it is no exaggeration to say that in many of the infective diseases a diagnosis which is made without a bacterioscopic examination is either mere guess-work or can only be made so late that the patient has suffered unnecessarily in health and the practitioner in prestige. In many cases, however, the investigation requires a considerable amount of technical skill and access to a well-equipped laboratory; the former may perhaps be possessed by the rising generation (for bacteriology is now an integral part of the medical curriculum), but it would be unfair to expect every medical man to add the latter to his already expensive equipage. But in many cases the diagnosis can be arrived at by very simple means—a few slides, cover-glasses, and stains, a good microscope (which ought to be considered as essential as a stethoscope), and a very moderate amount of technical skill will often enable the practitioner to arrive at correct diagnosis in a very short time. This little book is intended in the first instance to show exactly when this may be done, and to provide clear, succinct, and full descriptions of simple methods which may be employed. The descriptions of the operations which the practitioner can carry out for himself are mostly written in the imperative mood, and are intended to be referred to constantly and carried out, step by step, during the process. They represent the in-

structions which would be given by a teacher when watching a beginner making a simple bacteriological examination for the first time.

It cannot be too strongly urged that the practitioner should make the examination for himself whenever it is possible for him to do so. The report which is sent from a public laboratory may often be of very considerable value, but it must be remembered that the bacteriologist can only supply facts, and the inferences which may be drawn from those facts will largely depend upon a knowledge of the patient's clinical history and the method in which the material was obtained. The bacteriologist is too often in the position of a detective who has to unravel a mystery from observations made by other people, and has no opportunity of making investigations for himself. A bacteriological examination which is made by one person and interpreted by another, or which is made on material which has passed through more hands than one, loses much of its value; and an investigation made on the spot may be more valuable than one made by a bacteriologist of far greater experience at a distance.

The methods which are described in this little book are not in all cases the ideal ones, and in some cases they are somewhat different from those which are generally used, but they are simple and efficient. Of course, the simple examinations which are described here would frequently be supplemented by more complicated cultural ones by a trained bacteriologist. The methods described here have been taught in the post-graduate classes which were initiated some two years ago in the University of Birmingham; these have already been attended by about a hundred practitioners, who have found these methods of great assistance to them in their everyday practice. They have been selected so as to provide examples of some of the more important operations in constant use in the bacteriological laboratory. The author takes this opportunity of expressing his cordial thanks to Professor Leith for his kind suggestions as to the general scope of the book.

No apology will be made for the numerous repetitions which will be found in this book. They are essential to its scope, which is to give clear accounts of the processes with as little reference to other chapters as possible. In the majority of cases each section is complete in itself.

These instructions are followed by information as to the interpretation of the results which may be obtained ; and this information applies equally whether the medical man has made the examination for himself or has obtained it ready made from a public laboratory. It too commonly happens that practitioners feel themselves aggrieved because they get a negative report (as to the presence or absence of Widal's reaction) on blood taken during the first few days of an illness which turns out to be typhoid fever, or are inclined to discredit bacteriological examinations because diphtheria bacilli are found in throats which exhibit no membrane and clear up in a few days without serious symptoms.

In the second place, there are a good many cases in which the investigation had better be made in a public laboratory. In these the questions, of what to send, and how to send it, are fully explained. This a most important point. A bacteriologist is not a magician who is able to weave a spell if he has a small portion of his victim's anatomy to work upon ; and the materials *must* be taken in the proper way if his results are not to be useless or even misleading. This is well seen in the examination of the blood for bacteria. In many cases the blood is drawn in such a manner that it must necessarily be contaminated from the skin during the operation and is transmitted in vaccine tubes which were almost certainly not sterile before being filled. Under such circumstances the bacteriologist will probably report the presence of streptococci or staphylococci, and the practitioner who does not understand the fallacies of the examination may be led to make a diagnosis which will be disastrous to his own reputation and may be injurious to the patient.

Lastly, it need scarcely be said that this is not intended to be a substitute for any one of the numerous

excellent works on the science of bacteriology which are current at the present time. The practitioner is strongly recommended to supplement the very meagre details concerning the life-history and pathogenic action of the bacteria which are dealt with here by a study of one of these textbooks. Muir and Ritchie's admirable "Manual of Bacteriology," Crookshank's "Bacteriology and Infective Diseases," Hewlett's "Bacteriology," Klein's "Micro-organisms and Disease," McFarland's "Textbook upon the Pathogenic Bacteria," or Curtis's "Essentials of Practical Bacteriology" are all suitable for this purpose, and a perusal of any one of them will be both pleasurable and profitable to every medical man.

Several illustrations have been borrowed from sources mentioned in the text and for the loan of these the author wishes to express his best thanks to the respective authors and publishers. His best thanks are also due to Messrs. Baird and Tatlock, Swift and Son, Zeiss, Leitz, Hawksley, and Hearson, for kindly providing illustrations of apparatus made by them.

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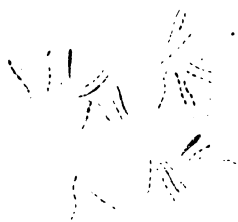








# PLATE I



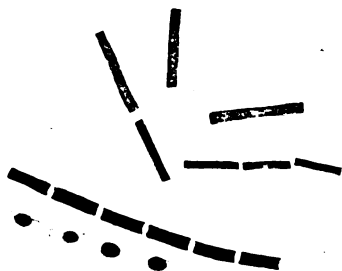
*Fig 1*



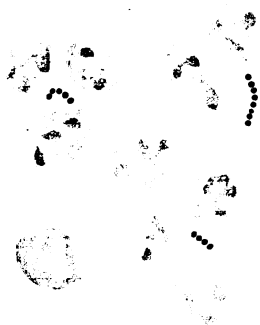
*Fig 2*



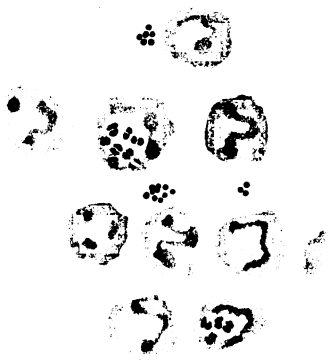
*Fig 3*



*Fig 4*



*Fig 5*



*Fig 6*

## DESCRIPTION OF PLATE I.

FIG. 1.—Diphtheria bacillus (long form) from a young culture on blood-serum. Löffler's blue;  $\times 1000$ .

FIG. 2.—Diphtheria bacillus (short form) and Hoffmann's bacillus. From young cultures on blood-serum. Löffler's blue;  $\times 1000$ .

FIG. 3.—Pneumococci in sputum from a case of pneumonia. Stained with dilute carbol fuchsin and thoroughly washed;  $\times 1000$ .

FIG. 4.—Anthrax bacilli. The lower portion of the field shows bamboo-like chains and spores, and is taken from cultures. Methylene blue;  $\times 1000$ . The portion showing spores is stained with carbol-fuchsin, decolorised by brief immersion in methylated spirit, and counterstained in methylene blue.

FIG. 5.—Pus showing streptococci. Stained by Gram's method, counterstained with eosin;  $\times 1000$ .

FIG. 6.—Pus showing gonococci and staphylococci. Stained by Gram's method and counterstained with dilute carbol-fuchsin;  $\times 1000$ .







PLATE II



*Fig 1*



*Fig 2*



*Fig 3*



*Fig 4*



*Fig 5*



*Fig 6*

## DESCRIPTION OF PLATE II.

FIG. 1.—Tetanus bacillus, from a culture. Stained with dilute carbol-fuchsin;  $\times 1000$ .

FIG. 2.—Tubercle bacilli. Stained with carbol-fuchsin and decolorised in dilute sulphuric acid; not counterstained;  $\times 1000$ .

FIG. 3.—Influenza bacillus in sputum. Some are contained within a leucocyte. Löffler's blue;  $\times 1000$ .

FIG. 4.—Plague bacilli from a culture. A short chain and some coccoid (involution) forms are shown. Löffler's blue;  $\times 1200$ .

FIG. 5.—Vibrio of Asiatic cholera. Dilute carbol-fuchsin;  $\times 1000$ .

FIG. 6.—Small colony of actinomyces, as it appears in pus. No clubs are visible. Gram's method;  $\times 600$ .



A HANDBOOK  
OF  
BACTERIOLOGICAL DIAGNOSIS  
FOR  
PRACTITIONERS.

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PART I.  
**APPARATUS AND PROCESSES.**

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THE BACTERIOLOGICAL MICROSCOPE.

The essentials which a microscope must possess in order to render it available for bacteriological work are:—

1. A *firm and rigid* stand and stage.
2. A firm, accurate, and delicate fine adjustment.

A microscope which possesses these may be made available for bacteriological work by the addition of the necessary parts, but one that is deficient in these respects is useless.

3. A convex and a flat mirror.
4. An Abbé's condenser and iris diaphragm.
5. Three lenses, a low power ( $\frac{1}{2}$  in. or better,  $\frac{3}{4}$  in.), a high power ( $\frac{1}{8}$  in. or thereabouts), and a  $\frac{1}{12}$  in. oil immersion.

If the practitioner already possesses a microscope

B

made by a reliable firm, and in good condition, this should be sent to a maker (not necessarily the maker of the microscope in question) or to a bacteriologist for an opinion as to whether or no it is sufficiently firm and



FIG. 1.—Bacteriological Microscope.

has a fine adjustment good enough to justify the addition of the other parts. If this is the case it should be fitted with an Abbé's condenser and an iris diaphragm; the cost should not exceed 30s. or £2. But

it is useless to do so unless the stand is sufficiently steady to carry an oil immersion lens: and it is useless to think of having an inefficient fine adjustment altered for bacteriological use.

There are now many microscopes which are sold at a

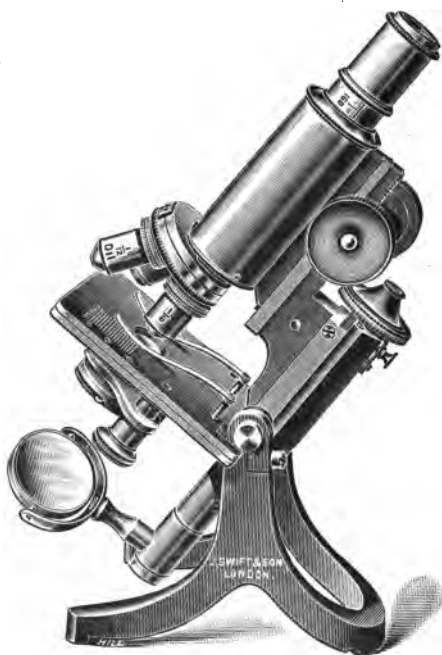


FIG. 2.—Bacteriological Microscope.

comparatively small price and which will answer every purpose. Of these Leitz's IIb stand, with the lenses above mentioned, a triple nose-piece (which is so great an advantage that it might almost be called an essential) and two eye-pieces costs about £13 and may be

B 2

highly commended. The stand is very firm and strong, the fine adjustment delicate, and the lenses altogether admirable. The only objection to this microscope is that it is rather heavy; this is an advantage for laboratory work, but it is a disadvantage for a medical man who may have to examine blood, &c., at the patient's bedside. Swift & Son manufacture a micro-



FIG. 3.—Portable Microscope.

scope of much the same type at about the same price; it has all the good points of the Leitz microscope and is somewhat lighter. The same firm also supplies a portable instrument, the cost of which (without lenses) is £5. This is very ingeniously made, and will carry an oil immersion lens quite well: it is perhaps the most suitable stand for a practitioner who may have to do

work at a distance, for it packs into a very small compass and is not heavy.

The cost of a  $\frac{1}{1\frac{1}{2}}$  in. oil immersion lens is, roughly speaking, £5. Beck supplies a good lens for £4, and Messrs. Gowlland of Selsey, Sussex, are now making one for £2 15s. 6d., of which I have formed a very high opinion after having examined a considerable number. The same firm also produces a microscope fitted with



FIG. 4.—Portable Microscope.

all requisites for bacteriological work for about £11, and this I believe to be quite efficient.

The cost of the other lenses may be put down at 30s. or less apiece. Leitz's No. 3 (about  $\frac{3}{4}$  in.) costs 15s., and his No. 6 (about  $\frac{1}{2}$  in.) costs 30s.; these are both extremely well adapted for bacteriological work.

With regard to eye-pieces, it is an advantage to have two, a No. 2 and a No. 4. Higher powers may be used, but it must be remembered that any increased



gain in magnification brought about by the eye-piece is attended by a loss of definition. The same applies to the increased magnification obtained by pulling out the draw-tube.

The total cost of a microscope may be put down at £15, and for this sum a thoroughly efficient instrument can be obtained; while one that will answer every purpose may be bought for decidedly less. The cost of adapting a good stand will be about £6 10s. or £7 (30s. or £2 for the substage arrangement and £5 for the lens) or less if a cheaper oil immersion lens is obtained.

It need scarcely be said that there are many admirable microscopes other than those mentioned above, which have simply been selected as favourable and low-priced instruments of English and Continental manufacture. Amongst others, the microscopes made by Beck, Ross, and Watson are all good, and reasonable in price.

## STERILISATION OF APPARATUS, ETC.

*Requisites* :—1. A thermometer graduated to 200° C.

2. A hot air steriliser.

*or* A cubical biscuit tin the soldering of which has been replaced by brazing. This must be mounted upon a tripod stand.

*or* A kitchen oven, preferably a gas oven.

3. A steam steriliser.

*or* A large kitchen steamer. This should be deep enough to contain a litre flask holding a funnel.

4. A large Bunsen burner or spirit lamp.

Bacteria and their spores are ubiquitous, and it is

necessary to sterilise all vessels and other apparatus, and all culture media before use. The methods which are adopted all depend upon the action of heat; chemical antiseptics are rarely used in the bacteriological laboratory for the sterilisation of apparatus, for it would be impossible to remove them completely, and the traces which might remain would prevent the development of those germs which we wished to



FIG. 5.—Slot Air Steriliser (simple form).

cultivate. Two chief methods are in use, sterilisation by *dry heat* and by *steam*; we exclude sterilisation by steam under pressure as this requires special and expensive apparatus.

**DRY HEAT** is used to sterilise all glass vessels (flasks Petri dishes, test-tubes, pipettes, &c.), cotton-wool, and metal instruments. The heat must be continued for at least an hour and must not fall below  $150^{\circ}$  C. as indi-

cated by the thermometer. Another method which is less reliable than the use of the thermometer, but which may be resorted to in an emergency, is to wrap the apparatus loosely in cotton-wool and to proceed with the heating (allowing the temperature to rise *gradually*) until the outer part of the wool is slightly singed over the whole of the exposed surface.



FIG. 6.—Steriliser.

The special steriliser which is used in the bacteriological laboratory consists of a copper or iron oven with double walls and perforated metal shelves. There is a hole in the top, which is fitted with a perforated cork, through which the thermometer passes. The oven is mounted on a stand and heated by means of a large Bunsen or Fletcher's burner.

An efficient steriliser may be made out of a cubical biscuit box, but it will not stand much usage unless the joints are brazed instead of being soldered: this can be done by any tinsmith. It is much better to have the the bottom of the box replaced by a sheet of copper, and a steriliser made in this way will answer every purpose and be fairly durable. A circular hole is cut through the centre of the lid and fitted with a cork bored so as to admit the thermometer. A false bottom or a shelf an inch or so from the bottom will keep the articles which are being sterilised from the heated surface; the false bottom may be made from a sheet of tin two inches longer in one of the sides than the bottom of the box. The extremities of the longer sides are to be turned down for a length of an inch, and several holes cut in the plate.

Lastly, the kitchen oven may be pressed into service if no other steriliser is at hand in an emergency. The apparatus to be sterilised is to be placed on a layer of cotton-wool on one of the shelves, and the temperature is observed by means of the thermometer, which should be thrust through the little window which permits of the regulation of the temperature. Or the heat may be continued until the cotton-wool is singed over the whole of the exposed surface. This method is very convenient for practitioners sending materials to a laboratory for bacteriological examination.

A gas oven is even more convenient, as the temperature can be regulated to a nicety.

All glass apparatus must be thoroughly cleansed and dried before sterilisation. The remaining steps differ somewhat in the different cases.

*Flasks* are plugged lightly with cotton-wool before being placed in the steriliser. *Bottles* may be sterilised

in the same way and are sometimes requisite for the transmission of fluids to a public laboratory for an examination which the practitioner is unable to carry out at home. They may also be sterilised by boiling. *Test-tubes* are treated in the same way as flasks. *Petri dishes* are wrapped round with tissue-paper or filter paper before being sterilised.

Before removing glass apparatus from the steriliser remember to let the temperature fall gradually, or the vessels may crack. In the case of a proper steriliser or of a biscuit tin heated by a burner the gas is turned out and the whole apparatus allowed to cool before the door is opened. In the case of a kitchen oven the best plan is to let the fire go out, or to open the door very gradually.

*Cotton-wool* is sterilised by being spread out in thin layers on the shelves of the apparatus, and the heat is continued until the outside is singed.

*Metal instruments* (knives, scissors, &c.) may be sterilised in the same way and at the same time. They should be wrapped loosely in cotton-wool, and should not be removed from their wrapping until the moment at which they are to be used.

STEAM is chiefly used for the sterilisation of culture media before use, and for the destruction of cultures when they are done with. The latter purpose, however, is accomplished more speedily and safely by the addition of a few drops of commercial formalin to each tube.

The proper steam steriliser consists of a metal cylinder with a perforated diaphragm six or eight inches from the bottom. It is enclosed in a thick layer of felt or other non-conductor of heat, and is provided with a lid. The space between the bottom and the

diaphragm is partly filled with water, which is boiled by means of a Bunsen flame or Fletcher burner, the apparatus to be sterilised being placed in the chamber above so as to be exposed to the steam.

An ordinary steamer (such as is used for cooking potatoes or fish) will answer every purpose. In pro-



FIG. 7.—Steam Sterilizer.

curing such a steamer for bacteriological use it is best to choose one that will accommodate a litre flask holding a funnel, as it is often a great convenience in the filtration of fluids which become solid on cooling to carry out the process in an atmosphere of steam.

Exact details of the way it is used will be given subsequently.

## PREPARATION OF CULTURE MEDIA.

Bacteria are grown in the same way as other plants. A gardener who wishes to grow a plot of a particular plant will first prepare a soil suitable for the growth of that plant and free it as far as possible of all seeds, roots, &c. He will then sow it with the seeds of the plant in question and do what he can to expose them to a suitable temperature. An exactly similar process is adopted when we wish to cultivate the smallest of all plants. The soil which we prepare is called the culture medium, and differs in the case of different bacteria; the process of freeing this soil from bacteria and their spores is called sterilisation and we ensure a suitable temperature by means of an incubator, the heat of which is kept constant.

The culture media which are used for special purposes are almost innumerable, but in the daily routine of the laboratory and for diagnostic purposes, broth, gelatin, agar-agar, and blood serum are all that are really necessary in the vast majority of cases. The blood-serum medium is difficult to prepare and can be replaced by ascitic agar in some cases. These media may all be bought from any firm of manufacturing chemists or from any bacteriological laboratory; and their purchase saves a great deal of work and is to be recommended for those who only wish to use them occasionally. They are sold in test-tubes which are kept sterile by being plugged fairly firmly with cotton-wool; this substance prevents the passage of bacteria as long as it is kept dry. The tubes are best stored in jars provided with tightly fitting lids, and it is an

advantage to place a shallow layer of a solution of perchloride of mercury (or other *non-volatile* antiseptic) in the bottom of each jar to prevent the medium from drying up. It is scarcely necessary to add that not the smallest trace of the lotion should be allowed to come into contact with the cotton-wool plug of the tubes. Or the medium may be kept from drying up by covering the tubes with india-rubber caps sold for the purpose.

BROTH is very easily made, and as it is the foundation of many other media, the practitioner is strongly advised to prepare it for himself.

*Requisites* :—1. Liebig's extract of meat.

2. Peptone.

3. Common salt.

4. A dilute solution of sodium carbonate—about 1 per cent., but the exact strength does not matter.

5. A large flask, a stirring rod, and a large glass funnel.

6. Test-tubes and cotton-wool plugs. The exact size of the tubes is unimportant, but 6 in.  $\times$   $\frac{3}{4}$  in. is convenient. The plugs are best prepared from wool which has been previously sterilised by dry heat, and should be fairly firm. The tube with the plug *in situ* must be sterilised by dry heat ready for use.

7. Litmus paper.

*Method*.—Take 1 litre of tap-water in the flask and add 5 grammes of Liebig's extract, 10 grammes of peptone, and 5 grammes of common salt, and boil until all are dissolved. Test the reaction by withdrawing a drop of the fluid on the stirring-rod and applying it to a piece of litmus paper. You will find that it is slightly acid. Now add some of the solution of soda drop by drop, testing after each addition, until the reaction of



the fluid is slightly alkaline.\* Boil the fluid for half an hour to coagulate any albumen which may be present.

Next filter the broth into a sterile flask, passing it through a double thickness of white filter or blotting paper, and plug the flask firmly with sterilised cotton-wool.

If the broth is to be used for the manufacture of gelatin or agar it is next sterilised in the flask, while if it is to be used as it is as a culture medium, it is decanted into tubes and then sterilised.

In decanting media into tubes be very careful not to get the plug wet and not to let any of the medium get on to the upper part of the tube; otherwise the plug will stick to the tube, and there will be some danger of bacteria from the air "growing through" the fluid contained in the interstices of the plug and contaminating the culture. Ordinary non-absorbent (brown) wool is better than the white absorbent wool, as it is less easily wetted.

The broth (and other culture medium after being melted) may be poured into the tubes in the following way. A sterilised funnel is united by a short length of india-rubber tubing to a piece of glass tubing drawn out to a point: the rubber tube is clipped by a spring clip or a pair of pressure forceps. The funnel is now mounted on a retort stand, filled with the medium, and covered over with a piece of glass. The cotton-wool plug is removed from a test-tube and the latter placed so that the glass tube attached to the funnel reaches nearly to the bottom. The clip is released and the

\* If during the neutralizing process too much alkali is added, then it is necessary to re-acidify with dilute hydrochloric acid and re-neutralize. The sodium chloride formed makes no *practical* difference in the medium.

requisite quantity of broth (enough to fill the tube to the depth of an inch and a half or two inches) is allowed to run in; the clip is then re-applied and the tube removed and plugged. This process is repeated until enough tubes have been filled.

The tubes and the broth which remains over (after having been poured back into the flask and the latter plugged with cotton-wool) are now sterilised. The vessels are placed in the steam steriliser and exposed to steam for half an hour on *three successive days*; this process is called intermittent sterilisation, and its rationale is very simple. The first steaming destroys all developed bacteria, and would sterilise the fluid entirely if no spores were present. In the interval between the first and second sterilisation most of the spores which may be present will develop into mature bacteria, and these will be killed by the second steaming. The third sterilisation is to kill off any bacteria which may not have developed from spores in the first interval. A very similar process is adopted by the gardener in freeing soil from weeds; the application of chemical weed-destroyers or a thorough hoeing will destroy developed plants but will not injure seeds which may be contained in the soil, and these processes are repeated, intervals being allowed to permit the development of the plants until they reach the stage in which they are vulnerable.

To recapitulate; mix the ingredients and heat until they are dissolved, render slightly alkaline, boil half an hour, filter. Then place in sterilised flask or into test-tubes and sterilise in the steam steriliser for half an hour on three successive days.

NUTRIENT GELATIN is broth which has been solidified by the addition of from ten to fifteen per cent. of gelatin;

the former amount is used in the winter, the latter in the summer. For general purposes  $12\frac{1}{2}$  per cent. may be used in all cases.

The special advantages of gelatin as a culture medium are two-fold. In the first place a great many organisms grow in or on it in a characteristic way, so that a bacteriologist may be able to identify the organism by inspection of the culture. This arises partly from the fact that some bacteria produce a ferment which digests gelatin just as pepsin does; these bacteria "liquefy" the gelatin, and the distinction between the bacteria which have and those which have not this property is very important for purposes of diagnosis. Further, some bacteria liquefy rapidly and others slowly, and this is another important point in the identification of a germ.

In the second place, the gelatin medium may be melted at a temperature (about  $25^{\circ}$  C.) at which bacteria are not killed. This fact is made use of in the isolation of bacteria from a fluid which contains several species by the process known as "*plating*." Suppose, for instance, that we find by microscopic examination that a specimen of pus contains two different species of bacteria (perhaps a bacillus and a coccus) and we wish to obtain the two organisms in pure culture so that we can ascertain their nature and properties. We take a tube of gelatin and melt it by placing it in warm water, and then inoculate the medium with a minute quantity of the pus.\* We then shake it so as to distribute the organisms throughout the melted fluid, and then pour the latter into a flat dish (Petri's plate) so that the gelatin flows out into a thin film and then sets. If

\* Another and preferable plan is to inoculate the medium first and to melt it afterwards.

our dilution has been properly made we shall have separated each organism from its neighbours, and each separate germ will grow up into a "colony" which will soon be visible to the naked eye. In all probability we shall be able to see that these colonies are of two kinds; one may liquefy and the other not, one may be coloured and the other colourless, one may be round and the other angular, &c. Samples of each sort of colony are then transplanted to fresh culture tubes and again incubated. An example of this process is given on p. 70.

A slight modification of this process enables us to make an estimate of the number of living bacteria which is present in a given fluid. To do this we have to follow out the above process, adding a *definite* measured quantity of the fluid to the culture tube of liquefied gelatin. The number of colonies which develop is counted, and this gives us the number of bacteria in the sample of fluid. For example, if one-tenth of a cubic centimetre diffused throughout a tube of melted gelatin and poured out into a thin film produced twenty colonies, it follows that one cubic centimetre of the fluid contained two hundred bacteria. This is a brief description of the essentials of the method adopted in the quantitative examination of water.

*Requisites* for the manufacture of gelatin:—

1. Broth.
2. Gelatin. (Coignet's gold label gelatin is best, but any good brand will do).
3. Dilute solution of sodium carbonate.
4. Litmus papers.
5. Flasks, stirring-rod, funnel, and plugged test-tubes as for broth.

*Method.*—Measure the broth and add to it  $12\frac{1}{2}$

C

grammes of gelatin for each 100 c.c.; allow to soak for an hour or more, and then heat until the gelatin is dissolved. Continue the heat and render the medium faintly alkaline just as was done in the preparation of broth. Now filter through a moistened filter paper. To avoid the setting of the gelatin during the filtration it is best to use a double jacketted funnel containing hot water, but if this is not at hand the whole apparatus (flask and funnel) may be placed in the steam steriliser (the lid being kept off to avoid the drops of condensed water which might otherwise fall into the funnel) or in a *warm* (but not hot) oven and left at a temperature of about 40° C. until the process is complete.

The gelatin which is made by the above process is sufficiently clear for most purposes. A more slightly medium may be made by clarification of the above by white of egg. To the medium (after neutralisation but before filtration) add the white of one egg for each 250 or 300 c.c. of fluid and shake thoroughly. Now boil in the steamer for half an hour and filter as before.

Test-tubes are filled with gelatin just in the same way as with broth, and the process must be carried out quickly to avoid solidification of the medium. Some of the test-tubes are allowed to cool in the vertical position, others lying in a sloping position so that the upper surface of the gelatin forms an ellipse some three inches long. The former tubes are inoculated by driving a straight platinum needle charged with the material containing the bacteria into the gelatin in the axis of the tube; cultures made in this way are called "stab-cultures." The gelatin "slopes" are inoculated by drawing the charged needle along the surface of the medium, care being taken not to plough it up; cultures made in this way are called "stroke-cultures."

AGAR is the name given to the dried strips of a Japanese sea-weed. It forms a jelly which differs from that containing gelatin in that it melts at a higher temperature; nutrient agar as used in the laboratory melts just below the boiling point of water and sets at about 40° C. This is an advantage in the cultivation of most pathogenic bacteria, for these grow (as a rule) best at or near the temperature of the body, the temperature to which they are exposed under natural circumstances; and at this temperature gelatin would melt. Agar is somewhat difficult to prepare unless the practitioner has an autoclave, and may be bought with advantage. But the following method is not very difficult, and, as agar is perhaps the most generally useful of all media, should be learnt.

*Requisites*:—1. Broth.

2. Agar-agar. This should be cut up into very small pieces with a pair of scissors, or may be bought in powder.

3. Solution of acetic acid. (Glacial acetic acid 2-4 c.c., water 500 c.c.).

4. A large beaker.

5. Other apparatus and materials as for gelatin.

*Method*.—Weigh out 2 grammes of agar for each 100 c.c. of broth to be used, and soak it in the dilute acetic acid for a quarter of an hour. Now strain off the acid and wash the agar in water until a small piece does not redden blue litmus paper when pressed upon it. Place the broth in a glass beaker and add the agar. Now place the beaker upon a piece of wire gauze upon a tripod stand, and apply a *small* Bunsen flame or spirit lamp; this must be placed so that the flame impinges on a point not far from the side of the beaker. As the fluid is heated it will rise and a continual circulation

will take place, so that the fragments will not stick to the bottom and cause it to crack. When all is dissolved the hot liquid must be carefully neutralised. It is then allowed to cool to about 50° C. and the white of an egg added for each 500 c.c. of fluid and mixed in thoroughly by being stirred with a glass rod. The whole is then placed in the steamer for an hour, at the end of which time the albumen should be completely coagulated. The beaker and its contents are then allowed to cool gradually, so that the coagulum (retaining all solid particles) may settle to the bottom before coagulation is complete. Perhaps the best method of accomplishing this is to place it in the oven (taking care that the temperature does not exceed 100° C.) after the fire has been raked out at night. In the morning the mass will be found to have solidified and there will be a coagulum at the bottom. The beaker is then inverted and the mass "turned out" just as a cook turns out a jelly, and the sediment is cut off with a sharp knife. This avoids filtration, which is very troublesome.

An alternative method is to filter the melted jelly through moistened filter paper or through two thicknesses of butter-muslin. It is necessary to keep flask and funnel in a steamer (the water of which is kept boiling vigorously) during the whole process, or the jelly will solidify in the outflow tube of the funnel.

The agar is again melted and placed in test-tubes; these are sterilised on three successive days and allowed to set in a sloping position. For certain purposes glucose, glycerine, &c., are added to the agar. The addition should be made to the melted medium just before it is poured into the tubes.

SOLIDIFIED BLOOD-SERUM is very difficult to prepare,

and is best purchased ready for use from a good laboratory. For clinical purposes it is chiefly used in the preparation of the diphtheria bacillus, for which, however, it may be replaced by the following medium, which is more easy to prepare.

ASCITIC AGAR :—*Requisites* :—

1. Clear ascitic, pleuritic, or hydrocele fluid.
2. A 10 per cent. solution of caustic potash.
3. Glycerine.
4. Other materials and apparatus as for agar. (Broth is not required, its place being taken by the pathological exudate).

*Method*.—Measure the fluid and add to it 2 c.c. of the potash solution for each 100 c.c. used. Mix thoroughly, pour a little into a test-tube, and heat. If the fluid does not coagulate proceed with the manufacture of the medium in the manner described below. If it coagulates add a little more potash, mix, and again test. The addition of the potash must be repeated until no coagulum forms on heating, all the albumen being converted into uncoagulable alkali-albumen.

The remaining steps of the process are the same as for ordinary agar, except that the neutralisation is omitted and 6 c.c. of glycerine per 100 c.c. of the medium is added whilst the agar is being dissolved.

POTATO tubes are in occasional use, and are easy to prepare. The process is as follows:—Take large and sound potatoes and scrub them thoroughly with a nail-brush under the tap. Peel them deeply enough to remove the eyes completely. Then cut them into cylinders a little less than  $\frac{3}{4}$  in. in diameter (if you are using  $\frac{3}{4}$  in. test tubes) and as long as possible; this is best done by means of a cork-borer, but they may be shaped by means of a knife if this is not at hand.



Then cut each cylinder in half by a cut running obliquely from end to end; the shape of each half should be exactly like that of the medium in a sloped gelatin tube. Place the halves in a large vessel of tap-water and allow them to soak all night; it is a good plan to use running water if possible.

After this has been done place each half (base downwards) in a test-tube, having previously inserted a small mass of absorbent cotton-wool and enough water to saturate it. Plug the mouth of the tube with cotton-wool and sterilise on three successive days.

### INOCULATION OF CULTURE MEDIA.

The method in which this is done varies greatly according to the end in view, and variations of the

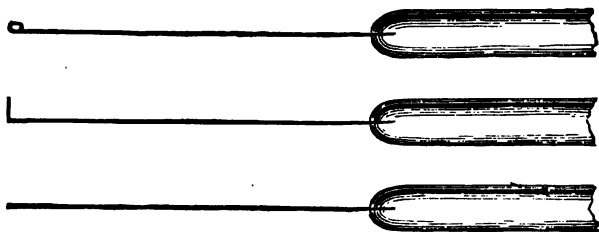


FIG. 8.—Platinum Needles.

process now to be described will be mentioned under their appropriate headings. We will suppose that we have to examine a specimen of pus, and wish to make a stroke-culture of agar and a stab-culture in gelatin. The following must be at hand:—

1. The pus.
2. A sloped agar tube and a stab gelatin tube.

3. A Bunsen's burner or a spirit lamp with a tall flame.

4. A pair of dissecting forceps.

5. Platinum needles. Each needle consists of a piece of platinum wire about three inches long mounted in the axis of a glass rod about six or eight inches in length. The wire should be just thick enough not to bend too easily. They are easily prepared. The rod is selected and the length of platinum wire is held in an ordinary pair of forceps. The end of the glass rod is held in the flame until quite soft; the end of the wire is then heated to redness, and pushed into the rod to the depth of about a quarter of an inch, taking care that it is

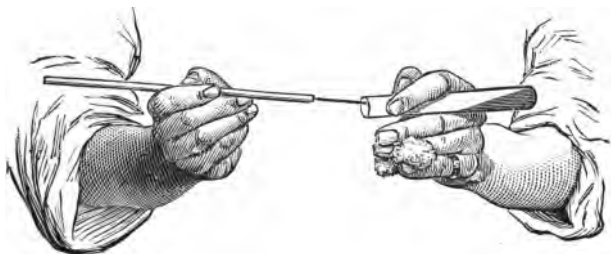


FIG. 9.

kept in the axis. The whole is allowed to cool and is ready for use.

For some purposes we use needles which terminate in a small loop, so that they will retain a drop of fluid. These are prepared in the same way as the straight needles, the free end of the wire being subsequently twisted round a French nail or other suitable object.

The method is as follows :—

1. Hold the culture tube you are going to inoculate first between the index and middle fingers of the left

hand, pointing the mouth of the tube slightly downwards (so that no dust shall drop into it) and to the right. Tubes of solid media should always be held in this position during inoculation; tubes of liquid media are held in a similar way, but of course their mouths must point upwards.

2. Put the projecting portion of the cotton-wool plug of the test tube into the flame so as to singe it; this is to destroy any germs which may have been deposited upon it from the atmosphere.

3. Sterilise the points of the forceps by passing them slowly through the flame and then use them to remove the plug. Place this between the ring and little fingers of the left hand and put the forceps down.

4. Take the platinum needle in the right hand, heat the whole of the wire to redness, and pass the lower three or four inches of the glass rod slowly through the flame. Remember that every portion of the needle which goes inside the tube must be sterilised in the flame. Allow the needle to cool; you should have found out how long this will take by a previous experiment.

5. Dip the tip of the needle into the pus; pass it into the tube until it reaches nearly to the bottom of the tube (now uppermost) and allow to rest upon the sloping surface of the medium; now withdraw it gently, allowing the tip of the wire to trail gently along the whole length of the sloped surface. Do not touch the medium with the glass shoulder of the needle.

6. Sterilise the needle as before. This step must never be forgotten.

7. Take the cotton-wool plug in the forceps, put it in the flame, and singe all parts of its surface. Then plug the tube while the wool is still burning. Label it.

To make a stab culture take the other gelatin tube and proceed as before until you get to step 5. When you have passed the needle into the tube drive it steadily into the medium, taking care not to deviate from the axis of the tube. Finish the process as before.

All this may seem involved. As a matter of fact it is very simple and need not take more than a minute to perform. But every step must be carried out, and the whole process must be learnt so thoroughly that it is performed automatically whenever a culture is made.

## INCUBATION OF CULTURES.

The limits between which bacteria can *live* are very wide; some grow best at one temperature, others at another, the limits for the great majority of organisms being about 16° C. and 40° C. In practice two temperatures are all that is used for ordinary work. The lower, or so-called "room temperature" is about 20° C. (68° F.) and is of most use for those bacteria which grow naturally outside the body, *i.e.*, as saprophytes. The higher, or body temperature, is about 37° C. (98·6° F.), and is the best temperature for the majority of germs which live within the body, *i.e.*, the parasites. It is obvious that gelatin cannot be incubated at this high temperature as it melts at 25° C. or thereabouts; but all other media are available.

The term "room temperature" must not mislead the practitioner, for the temperature of most rooms is rarely constant at or near 20° C. for periods sufficiently long to permit of its use for incubating bacteria. In the laboratory we use an incubator, the temperature of which is regulated by means of an automatic regulator,

and remains constant for long periods whatever be the external temperature. It is hardly necessary for the practitioner to purchase one of these. Careful search in the house will usually reveal some cupboard or

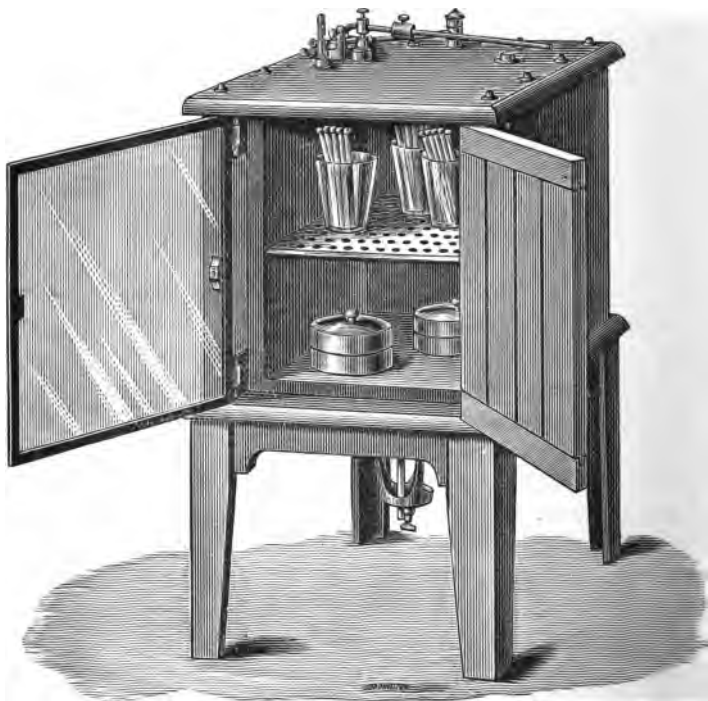


FIG. 10.—Incubator.

corner in which the temperature will remain sufficiently near  $20^{\circ}$  C. for a sufficiently long period; it is more important that it should not rise above  $22^{\circ}$  C. than that it should not fall below  $18^{\circ}$  C., as the former tempera-

ture may melt the gelatin, while the latter will only delay the growth of the colonies. It will probably be necessary to find one such place in the hot weather (*e.g.*, the cellar) and another one in the winter (*e.g.*, a cupboard not far from the hot-water pipes).

It is necessary that cultures which are being incubated should be kept *in the dark*, as light is inimical to the development of nearly all bacteria.

Incubation at the body temperature presents more difficulty. An incubator is almost essential when much work has to be done, and of these Hearson's is by far the best. The smallest size costs about £6 10s. and is a thoroughly reliable and durable affair. Cheaper instruments are also procurable; the Edinburgh incubator (Alex. Frazer, 22, Teviot Place, Edinburgh) costs about £4 10s. complete. Foreign incubators can be obtained at an even lower price, but are not very durable.

Much can be done without the use of so expensive an apparatus if the practitioner can find a room in which the temperature keeps approximately constant throughout the twenty-four hours. A tin biscuit box (or any other metal box) is covered with cotton-wool on the top and sides, the bottom being left bare, and mounted on a tripod stand. It is heated by means of an ordinary night-light (two may be necessary if the weather is cold) shielded from draughts by means of a wide lamp-chimney or a tin cylinder made out of an ordinary canister. The temperature is observed by means of a thermometer projecting through a hole in the lid, and the night-light raised or lowered until the temperature reaches the desired figure. The whole apparatus should be placed on a metal tray containing a small quantity of water and put in the middle of the

floor and away from any inflammable materials. This will be found to answer admirably and can easily be fitted up in an emergency.

It would be better to use a tin box specially made for the purpose and having a door at the side and a perforated false bottom so that the culture tubes do not rest directly on the metal exposed to the flame. This latter had best be made of copper.

If the practitioner is fortunate enough to possess a conservatory which is kept at a temperature approximating to that of the body, this will serve admirably. The culture tubes must be kept in a box which will exclude light.

In the absence of this a cupboard near the kitchen fire or the hot water cistern may be found that will answer the purpose; a thermometer should be placed in it and examined from time to time throughout the day, and if the temperature does not fall below  $30^{\circ}$  nor rise above  $40^{\circ}$  it will serve at a pinch, though a temperature which is more constant near  $37^{\circ}$  is desirable. It has to be remembered that we are not now speaking of the incubation of cultures for purposes of research; we are dealing with methods of cultivation which are necessary for diagnostic purposes, and for these it is usually sufficient if the temperature remains nearly constant at the proper point for some eighteen hours.

The author once succeeded in making a diagnosis in a case of supposed diphtheria by the following method. The tube of medium was inoculated from the throat and placed in a jam pot which was partly filled with water at  $37^{\circ}$  C.; a thermometer was also inserted and the whole placed near the fire. It was watched from time to time and moved near the fire if the temperature showed signs of falling, and *vice versa*. Next morning

there was an excellent growth and the diagnosis was made with certainty.

Lastly, the author has heard of a practitioner who was in the habit of incubating cultures at the body temperature by carrying them in an inner pocket during the day and taking them to bed with him at night!

## METHOD OF EXAMINING CULTURES.

*Requisites.*—1. Clean slides and cover-glasses. (These must be the thinnest in ordinary use, *i.e.*, No. 1).

2. A platinum needle. (Straight or loop).

3. A Bunsen's burner or a spirit lamp with a tall flame.

4. The stain to be employed (see p. 37).

5. Canada balsam dissolved in xylol. This should be bought ready for use.

6. A pair of dissecting forceps.

7. Strips of white blotting- or filter-paper.

*Process.*—1. Sterilise the needle and place a *small* drop of water (preferably distilled) in the centre of a clean slide.

The disadvantage of using tap-water arises from the fact that it leaves a deposit of salts when it has dried, and these salts make the film "dirty." The amount of distilled water required is very small, and sufficient can be obtained by holding a cold tumbler or saucer in the steam from a kettle.

2. Take the culture-tube in the left hand between the index and middle fingers with its mouth directed to the right and (in the case of a culture on a solid medium) slightly downward.



3. Burn the surface of the plug in the flame. Remove the plug with the forceps (previously sterilised by being passed slowly through the flame) and place it between the ring and little fingers of the left hand. Lay the forceps down.

In cases where you are examining the culture for diagnostic purposes only, and do not care if it becomes contaminated during the process, it is unnecessary to take these precautions. The cotton-wool plug may then be removed with the fingers and laid down on the table. As a matter of fact very few cultures do become contaminated even if no precautions are taken.

4. Sterilise the needle in the flame and allow it to cool.

5. Now introduce the needle into the tube and take up a *small* portion of the growth, taking care not to scrape up the surface of the medium as you do so. Most beginners fall into the mistake of taking up far too much of the growth, and preparing a film which is spread so thickly that the individual bacteria cannot be distinguished.

6. Take the plug up in the forceps, burn its surface in the flame, re-plug the tube and lay it down.

7. Stir the droplet of water which has been deposited on the slide with the tip of the needle so that the bacteria which it carries are mixed with the water. Now spread out the emulsion thus produced so as to form a patch about half an inch in diameter. If it does not spread out uniformly it is a sign that the slide is not clean.

8. Sterilise your needle.

9. Allow the film to dry spontaneously. If you have spread it out sufficiently this will take a very short time.

10. Fix the film by passing the slide slowly through

the flame once or twice. This coagulates the albumen present and the bacteria are now fixed down so firmly that they will not be removed by subsequent washing. The exact amount of heat which should be used cannot be stated, as it varies according to the thickness of the slide, &c., and can only be determined by practice. It may be estimated roughly by pressing the finger upon the upper surface of the slide close to the film, but not touching it. The slide should be just uncomfortably warm to the finger, but not hot enough to burn it.

11. Filter a few drops of the stain on to the surface of the film and allow it to act for the requisite time. Exact details will be given in each case.

12. Next wash the slide under the tap, blot it with clean white filter paper, taking care to avoid rubbing, and heat it gently over the flame until *absolutely* dry.

13. Place a drop of balsam on the film and apply a clean dry cover-glass.

The preparation is now ready for examination.

This also is a process which sounds more complicated than it really is. The steps are readily learnt, and the whole process (excluding the time spent in staining, which may be long or short), does not take more than two or three minutes.

Most bacteriologists make their films on cover-glasses and not on slides. The steps are just the same, except that the cover-glasses, being much thinner, naturally require much less heating to fix the film; they are passed *rapidly* through the flame three times. It is a great advantage to use Cornet's forceps in working with cover-glasses. These forceps are self-retaining, and hold the cover-glass in a horizontal position so that stains can be poured upon them whilst the forceps are resting on the table. But the staining can be carried

out equally well in a watch-glass, and the cover-glass may be held in dissecting forceps.

It is far easier and more satisfactory in every way to make the films on the slides. Beginners will find that they will break large numbers of cover-glasses (which must be thin), drop more on the floor, and will be in constant doubt as to which is the film side. With slides these difficulties do not occur, and the use of forceps is quite unnecessary.

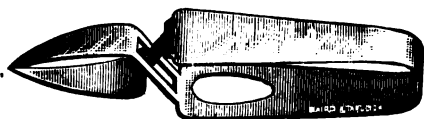


FIG. II.—Cornet's Forceps.

## GRAM'S METHOD OF STAINING.

The method of staining described above is available for all organisms, and therein consists its advantage. But other things than bacteria are stained; pus-cells, fragments of tissue, debris, &c., will all be coloured, and may obscure, or even be mistaken for, bacteria. Gram's method possesses the enormous advantage that by its use the bacteria are coloured, while other structures (with the exception of particles of keratin and dividing nuclei) are not. Hence in a film stained in such a way the bacteria are very distinct.

Gram's method possesses another advantage. It is a *selective stain*. Some bacteria retain the stain, whilst others do not, and this fact is of great value in diagnosis. The diphtheria bacillus, for instance, stains

when treated in the way we shall describe, and if an organism which presents the character of that bacillus does not stain by the process it must be of some other species. We shall append a table of the most important pathogenic bacteria which stain and which do not stain in this way.

*Requisites.*—1. Aniline gentian violet, or carbol-gentian violet (see p. 38).

2. Gram's iodine solution (see p. 39).

3. Absolute alcohol or methylated spirit.

*Process.*—1. Spread, dry, and fix the film in the way described above. Stain for two or three minutes in the aniline gentian violet or carbol-gentian violet.

2. Rinse in water to remove excess of stain and flood with the iodine solution, and allow the latter to act for a minute.

3. Wash off the iodine solution with alcohol, and continue the application of the latter until no more colour comes away. It is best to pour a little alcohol on the slide and rock the latter from side to side for a minute or so, then to pour off the alcohol and add a fresh supply, and continue this until the alcohol comes off colourless.

Dry and mount as before.

The following important bacteria *stain* by Gram's method :—

Staphylococci.

Streptococci.

Pneumococci.

In fact all important pathogenic cocci except the coccus of Malta fever and certain diplococci enumerated below :—

The bacillus of anthrax.

„ „ diphtheria.

The bacillus of tetanus.

„ „ tuberculosis.

„ „ leprosy.

The actinomycosis fungus.

The following important pathogenic bacteria *do not stain* by Gram's method :—

The gonococcus.

Diplococcus intracellularis meningitidis (Weichselbaum).

Still's diplococcus of posterior meningitis.

The pneumo-bacillus.

The bacilli coli communis.

The bacillus of glanders.

„ „ typhoid fever.

„ „ influenza.

„ „ plague.

„ „ soft sore.

The vibrio of Asiatic cholera.

## EXAMINATION OF FILMS. USE OF MICROSCOPE.

Daylight is the best illuminant for microscopic work, and the light reflected from a white cloud opposite the sun is best of all. Direct sunlight is useless, but the light obtained from a ground glass window on which the sun is shining is very good.

For work at night the light from an incandescent gas-burner at a distance of two or three feet is excellent, but an ordinary paraffin lamp will answer quite well.

Having arranged for a suitable source of light turn the flat mirror uppermost and move it about until

a beam of light is thrown on to the condenser. Remember :—

In examining *stained* specimens use a *large* diaphragm.

In examining *unstained* objects use a *small* diaphragm.

You are now about to examine a stained specimen. Place the slide on the stage, putting the stained film in the centre of the aperture, and turn on the low power. Look down the eye-piece and move the mirror about until the field is brilliantly illuminated. Focus the microscope (using the coarse adjustment) until the image is clearly defined. Now move the slide about until there is a deeply stained area in the centre of the field. This area will not necessarily be the best for examination with a higher power, but it will serve to catch the eye when focussing the lenses which focus at a short distance from the object.

Now turn on the high power (the  $\frac{1}{6}$  in.). Remember that the "working distance" of all lenses is necessarily less than their focal distance, and that a  $\frac{1}{6}$  in. lens focusses at a distance from the object which is decidedly less than one-sixth of an inch; so also with the other powers. Lower the lens until it almost touches the object and screw up the sub-stage condenser as high as it will go. Look down the microscope and focus slightly upwards, using the coarse adjustment, until you catch a glimpse of colour; then focus very slowly until the object is sharply defined.

After a little practice you will be able to focus downwards on to the film, keeping a sharp look out for the first appearance of colour, but for beginners the foregoing method is easier and safer.

Study the object with the high power, and move it about until you find an area where the bacteria are neither too thickly nor too thinly spread and are well

stained. Make out as much of their appearance as you are able to do with this power. Very much can be done; tubercle bacilli, gonococci, and many other bacteria may be recognised with this power, and the peculiar arrangement of diphtheria bacilli can be seen.

Apply the clips to keep the slide in place.

Now raise the tube of the microscope for a short distance, using the coarse adjustment, and place a small drop of cedar oil on the centre of the cover-glass. Lower the tube (using the coarse adjustment) until the nozzle of the lens touches the drop of oil; then put your head on a level with the stage and continue to focus downwards, going very carefully, until the lens *almost* touches the cover-glass. Next look down the microscope and focus upwards, using the fine adjustment, until you begin to see colour; then go more slowly until the film is well defined.

Beginners are strongly urged to adopt this method of focussing an oil immersion lens until they have acquired a considerable amount of practice. It takes a little time, but this is well repaid by the absence of all danger or injury to lens and cover-glass. After a time you may lower the lens until it touches the oil and then look down the microscope and continue to lower it with the fine adjustment.

After use, wipe the front of the immersion lens with a soft silk handkerchief kept specially for the purpose, and put the microscope back into its case. If oil or balsam should get dried on the lens wipe it with a handkerchief just moistened with xylol or pure turpentine and then wipe quickly with a dry handkerchief. Never dip the point of a lens into xylol or alcohol. Never remove the front combination of an oil immersion lens for cleaning or any other purpose.

## STAINS.

The following stains are all that is really necessary for the vast majority of purposes:—methylene blue, *basic* fuchsin, gentian violet, thionin, and water-soluble eosin. Bismarck brown may also be obtained. Ten grammes will last the practitioner for a long time, and this amount costs from 7*d.* to 1*s.* They should be of Grüber's make, and can be obtained from R. Kanthack, Berners St., W.; Messrs. Baird and Tatlock, Hatton Garden and Renfrew St., Glasgow; A. Frazer, Teviot Place, Edinburgh; and from Messrs. Southall or Philip Harris, Birmingham. Other firms will also supply stains, but Grüber's should always be specified.

They are conveniently kept in a saturated solution of absolute alcohol. The following formulæ are the most useful:—

1. *A saturated watery solution of methylene blue.*—This does not keep very well, and a fresh amount should be prepared after a month. It is mostly used for staining blood-films. Löffler's blue will serve every purpose in bacteriological work.

2. *Löffler's methylene blue* is prepared by adding 30 c.c. of saturated solution of methylene blue (alcoholic) to 100 c.c. of a 1 in 10,000 solution of caustic potash.

The potash solution is prepared thus:—Take 1 c.c. of a 10 per cent. solution of caustic potash and make up to 100 c.c. with water; shake thoroughly and pour away 90 c.c.; make up to 100 c.c. with water, and again shake. A sufficiently close approximation is made by adding 1 minim of the 10 per cent. solution to 2 oz. of water.

This stain keeps fairly well.



3. *Carbol-fuchsin* is made by adding a saturated alcoholic solution of fuchsin to carbolic acid lotion (1 in 20) until the fluid has lost its transparency. This keeps well.

4. The above stain diluted with four or five times its volume of water. Label "*dilute carbol-fuchsin.*"

5. *Aniline gentian violet*, which is prepared as follows:—

First prepare aniline oil water by shaking water (preferably distilled) with more aniline oil than it will dissolve; a milky emulsion will result, and this must be allowed to settle for a short time. Then filter it through a double thickness of filter paper which has been previously moistened with water.

To 9 parts of the solution thus obtained add 1 part of saturated solution of gentian violet.

This solution keeps badly, and it is of great importance that it should be freshly prepared, as very important inferences are drawn from results obtained with it. The following keeps better, and answers every purpose.

*Carbolic gentian violet*.—(A substitute for aniline gentian violet).

Add 1 part of saturated alcoholic solution of gentian violet to 9 parts of a 1 in 20 carbolic lotion.

6. *Carbol-thionin* is made by adding 1 gramme of thionin to 100 c.c. of a 1 in 40 solution of carbolic acid.

This stain keeps fairly well, but it must always be filtered immediately before use, as crystals which may have a most delusive resemblance to long slender bacilli are frequently deposited in it. A similar formation of crystals also occurs if the stain be allowed to dry on the slide.

7. *Eosin* is used in a 4 or 5 per cent. watery solution.

This keeps well. Red ink (slightly diluted) will answer most purposes.

Stains should be filtered before use. Where much work is to be done it is convenient to keep them in bottles which are closed with a perforated cork through which a small glass funnel is placed. A filter paper is kept permanently in this funnel, and the stain is filtered directly on to the slide or cover-glass.

*Gram's iodine solution* may be mentioned here, though it is not a stain. It consists of a solution of iodine 1 part, iodide of potassium 2 parts, water 300 parts. It keeps indefinitely.

## CLEANING SLIDES AND COVER-GLASSES.

Slides and cover-glasses must be absolutely clean when used in the bacteriological laboratory; it is especially necessary that they should be free from the slightest trace of grease, for this will prevent fluid from spreading out into a thin and uniform film.

Slides are best cleansed by dropping them one at a time into strong nitric or sulphuric acid, and allowing them to soak for an hour or more. They are then washed in running water for another hour, soaked in strong ammonia for an hour, and kept in alcohol. As methylated spirit is not so good for this purpose the use of alcohol is rather expensive; but it is not absolutely necessary, and the slides may be stored in a solution of ammonia (about 1 in 10) until required. When about to be used they are to be wiped dry with an old linen handkerchief kept specially for the purpose. This handkerchief should be as old as possible, and should have been washed until it has begun to fall to pieces.

When no properly cleaned slides are at hand the following method may be adopted, though it is not so good. Dip the end of a clean handkerchief in strong spirit (absolute or rectified) and wipe the slide with it, using a considerable amount of friction. Now dry it with the special handkerchief mentioned above, heat it thoroughly in a smokeless flame, and allow to cool completely. Spread the film on the surface which was exposed directly to the flame.

Cover-glasses are cleaned in the method advised for slides, and should be stored in strong alcohol smelling strongly of ammonia. They are wiped with the special handkerchief immediately before use.

When cover-glasses are to be used for covering films spread upon slides (as is generally the case if the method recommended in this book is adopted) it is quite sufficient to wipe them carefully with a clean handkerchief moistened with spirit and then to dry them.

After slides or cover-glasses have been cleaned the utmost care must be taken that they do not come in contact with the skin, or a thin film of grease will be deposited upon them.

## PIPETTES.

Glass pipettes for the collection of pathological fluid for bacteriological examination are in daily use in the laboratory, and are very necessary for practitioners who wish to send fluids for examination. They are readily made from a piece of quill glass tubing, and a few should always be kept in stock against emergencies.

The pipette consists of a bulb about half an inch long, each end of which is drawn out into a narrow tube at least six inches long, tapering gradually to the extremities (fig. 12, *a*). To make such a pipette take a piece of glass tubing about six inches long and a quarter of an inch wide, and heat it in a luminous gas flame at a point half an inch or so from the centre. Continue the heat until the glass is thoroughly softened over at least half an inch of its length, turning the tube round all the time; then remove it from the flame and draw the two ends apart with a steady uniform pull, so that the heated portion draws out into a capillary tube several

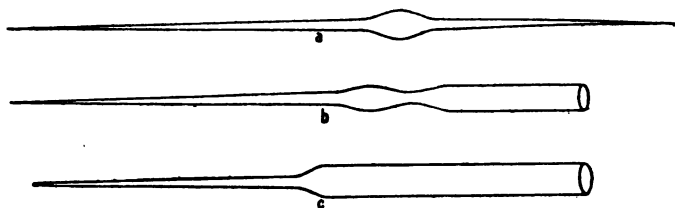


FIG. 12.—Pipettes.

inches in length. Repeat the process at a point about half an inch from the tapering end of the larger portion of the tube; heat the bulb and then seal off both ends of the capillary portion before the bulb cools.

The pipettes are necessarily sterile, having been drawn out of partially melted glass, and they will remain sterile indefinitely. Of course the exterior of the glass will become contaminated, and it should be passed through the flame before use.

The ends of the tube being sealed up while the bulb contains heated air, it follows that the bulb will contain a partial vacuum on cooling. This fact is made use of

in the collection of specimens. Suppose, for instance, we wish to take some blood from a heart at a post-mortem examination for investigation at a distance. A point on the surface of the heart is first seared with a hot iron to destroy any germs which might be present, and the end of the pipette (still sealed) is thrust through into one of the cavities. It is then broken off by dexterous pressure against the heart-wall, and the pipette will fill slowly with the blood. Another method is to break off the tip of the pipette and to warm the bulb before making the puncture. The fluid will rise as the bulb cools; or both ends may be broken up and the fluid drawn into the bulb by gentle suction.

Under any circumstances both ends of the pipette must be sealed up in a flame (the flame of a wax match will answer at a pinch) and the tube labelled.

Another variety of pipette which is much used for the transmission of blood, for the purpose of testing the serum reaction for typhoid fever, is drawn out to a point at one end only, the other being left wide and separated from the bulb by a constriction (fig. 12 *b*). The open end should be loosely plugged with cotton-wool, and serves as a mouthpiece. The manufacture of these pipettes presents a little difficulty, but a small amount of practice will enable the practitioner to turn out a perfectly serviceable one on occasion. These pipettes are not so suitable as the others for the transmission of fluids for cultural examination or injection into animals.

## PART II.

**DIAGNOSIS OF CERTAIN DISEASES.**

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DIPHTHERIA.

Diphtheria is a local disease with general symptoms. The local symptoms are due to the local action of the bacillus which causes the disease, while the general symptoms are due to the toxin or poison which they produce and which is carried in the blood-stream to the brain, heart and other organs. Now the local symptoms are comparatively unimportant, and it is to the general symptoms caused by the toxin that diphtheria owes the greater part of its high mortality. Diphtheria antitoxin neutralises this toxin (much in the same way as an alkali neutralises an acid) and prevents it from harming the vital structures; but it does *not* repair the harm that the toxin has done. It is obvious, therefore, that we must not make our diagnosis of diphtheria from the general symptoms if the antitoxin treatment is to do any good. The diagnosis is to be made from the local symptoms, and this is what we can rarely do by ordinary clinical methods at a stage sufficiently early to get the full value of the antitoxin treatment.

The practitioner has a choice of two methods. He may inject all patients who suffer from sore throats which present the slightest resemblance to those seen in diphtheria, or he may employ bacteriological methods

of diagnosis. The former method may be applicable in an epidemic of diphtheria, but suspicious throats are common and antitoxin expensive. In most cases it is necessary to have recourse to the second method.

Most sanitary authorities have now recognised that it is their duty and privilege to provide for the bacterial investigation of supposed diphtheria free of charge to doctor and patient, and supply outfits to be used for taking the material and transmitting it to the laboratory. When the practitioner lives within easy reach of the laboratory (so that the swabs may reach it quickly) it is his bounden duty to avail himself of the opportunity thus afforded of getting a free opinion from a specialist.

But the case of a practitioner living at a distance is somewhat different. Suppose the patient is seen on the first day of the illness, an unusual occurrence except in an epidemic. The swab is taken, dispatched by post, and reaches the laboratory on the second day. It is inoculated, and the culture is incubated and examined on the third day, the result reaching the practitioner about noon on that day. Now the mortality of diphtheria which is treated with antitoxin on the first day is very small, certainly less than five per cent., while the mortality in cases in which its use is not commenced until the third day is much higher, probably from ten to fifteen per cent., or even higher. In other words, from 5 to 10 patients out of every 100 lose their lives if the doctor waits for the result of the bacteriological examination. It is therefore highly advisable that every practitioner should provide himself with a bacteriological microscope, and should *at least* examine a film prepared directly from the swab, and stained in the manner described below. He should *also* make

cultures or send a swab to the laboratory for examination.

Swabs and outfits are provided by the laboratory where the examination is made, or can be bought from most manufacturing chemists and instrument makers. A swab consists of a steel or copper (aluminium would be better) wire, the extremity of which is covered drumstick fashion with a tightly fitting roll of cotton-wool. The other end is pushed through a cork, and the whole is contained in a stout glass tube. It is sterilised before use. These swabs may be readily made at home. A test-tube is fitted with a good cork through which is passed a stout steel knitting needle. This should be long enough to pass nearly to the bottom of the tube when the cork is in place, and the end which is to be outside the tube should be cut off short. The other must be roughened by a few strokes of a file. A small piece of cotton-wool (unmedicated) is then held between the thumb and finger of the left hand, transfixcd with the roughened end of the wire, and twisted round it. The swab is now placed loosely in the tube and sterilised by dry heat (see *ante*, p. 7). It is allowed to cool in the steriliser, and the cork is pushed home into the tube as soon as it is cold enough to handle. These swabs will keep indefinitely, and a stock of them should always be kept at hand. After use the cotton-wool should be burnt off in a Bunsen's burner or spirit lamp, and another piece applied and the whole re-sterilised.

If a practitioner should see a supposed case of diphtheria when he is unprovided with a swab he can readily extemporise one which will answer sufficiently well out of some cotton-wool (non-medicated), a wooden skewer or pen-holder, and a glass phial. The wool is



wrapped round the tip of the skewer, and (after the swab has been taken) the latter is placed in the phial in such a position that the cotton-wool does not touch the glass; the place between the skewer and the neck of the bottle is plugged with cotton-wool. It is not *absolutely necessary* to sterilise the swab, although it is a very great advantage to do so if time permits.

The method of *taking the swab* is of great importance, and must be carried out in full detail. It is necessary that the patient should not have had an antiseptic gargle or application for *at least* two hours previously. It is also advisable to allow him to drink some beef tea or boiled water (*not milk*, for this may contain certain bacilli which closely resemble those of diphtheria) immediately before the process. This will serve to cleanse the parts.

*Requisites.*—1. A good light.

2. The swab in its tube.

3. A tongue depressor. The form which is hinged so as to bend at a right angle is most convenient.

4. A vessel containing antiseptic lotion or boiling water.

*Method.*—1. Place the patient so as to face the light. If a small child he should be held on his nurse's lap.

2. Loosen the cork in the tube so that the swab may be withdrawn with one hand and place it at a convenient spot on your right side.

3. Get the patient to open his mouth, insert the tongue depressor (held in the left hand) and get a clear view of the area chiefly affected. Do not proceed with the process (if it can be avoided) until you have done this.

4. Take the cork between the finger and thumb of the right hand and pass the swab into the patient's

mouth, taking great care not to touch his lips, tongue, or palate. Press it firmly against the area which you wish to examine and rotate it between the finger and thumb so as to remove some of the secretion, and, if possible, some of the membrane. Withdraw the swab, again taking care not to touch any part of the mouth, and replace it in the tube.

5. Withdraw the tongue depressor and place it in the antiseptic lotion or boiling water.

6. Push the cork home into the tube.

Method of examining the swab.

This may be carried out by means of stained films prepared directly from the swabs, or by means of cultures. The former method is less useful than the latter, but we shall consider it first, as it can be performed by anyone who possesses a microscope carrying a  $\frac{1}{18}$  in. oil immersion lens, and often gives valuable information. Moreover, it does not take long and but little delay is caused.

*Requisites.*—1. Clean slides and cover-glasses.

2. Stains—Löffler's blue or carbol-thionin, and aniline gentian violet.

3. Gram's iodine solution and alcohol—methylated spirit will do.

4. Strips of white filter- or blotting-paper.

5. Balsam.

*Method.*—Prepare a film in the following way:—Rub the swab on the middle of a clean slide so as to spread some of the secretion into a thin layer on the surface. Allow it to dry, and fix it by passing it slowly through the flame until the upper surface is just too hot to prevent your pressing your finger upon it in comfort. Allow it to cool.

Now filter a few drops of Löffler's blue or carbol-

thionin on to the film and allow it to act for 2 minutes. Wash under the tap.

Dry by pressing carefully with strips of blotting-paper and then in the flame. Place a drop of balsam upon the film and apply a cover-glass.

Prepare a second film and stain by Gram's method (p. 32).

The films are now examined microscopically (see p. 34). We shall defer the description of the points upon which a diagnosis is to be based until we deal with the examination of cultures.

### CULTURAL METHODS.

The diphtheria bacillus grows best at or near the body temperature (about 37° C.) and flourishes on almost all culture media. But agar is scarcely ever used in growing it for diagnostic purposes: this medium serves well for the cultivation of a great many organisms, some of which are almost always present in the mouth, so that cultures made upon it are usually very impure. We use a medium which permits the development of the diphtheria bacillus and inhibits that of most other organisms. The best are ascitic agar and solidified blood-serum.

The method in which the medium is inoculated is as follows:—The tube of culture medium and the tube containing the swab are held side by side between the index and middle fingers of the left hand, the mouths of the tubes pointing to the right and slightly downwards. The plug of the culture tube is then singed, removed by means of a pair of forceps, and placed between the ring and little fingers of the left hand. The

cork and wire of the swab tube are now withdrawn and the cotton-wool plug is inserted into the culture tube and passed onwards until it reaches the sloped surface of the medium. It is then rubbed gently on the latter and twisted round and round so that every part of the swab may come into contact with the medium. If there is a piece of membrane special care should be taken to see that it is rubbed on the surface, for it is here that we are most likely to find the bacilli. The swab is now withdrawn and replaced in the tube, and the cotton-wool plug of the culture tube singed and replaced.

The tube thus inoculated must now be incubated for about 18 hours at a temperature not exceeding 37° C., and is then ready for examination.

Hewlett has suggested a useful method which may be carried out without any special apparatus, the white of a hard-boiled egg being used as the culture medium. Take a fresh egg and boil it for ten minutes or more and allow it to cool. Now take a narrow-mouthed wineglass (or a wide-mouthed bottle, which is better) and rinse it out with perchloride of mercury lotion. Sterilise a knife by passing it slowly through the flame, and cut off the top of the egg, care being taken not to cut into the yolk. Invert the egg into the wineglass (which must be narrow enough to prevent the egg from dropping down into it) taking care that none of the lotion touches the cut surface. This is the culture medium, and it is sterilised ready for inoculation. At a pinch it may be incubated in a warm corner near the fire, near the hot water cistern, or other warm place.

## EXAMINATION OF THE CULTURES.

I. *Naked eye*.—Each living diphtheria bacillus which has been deposited upon the surface of the culture medium and kept at a suitable temperature will develop into a colony of bacilli; and these colonies are fairly distinctive, being different from those which are formed by most other organisms. The expert bacteriologist can often give an accurate guess as to the presence or absence of diphtheria bacilli by mere inspection of the cultures. The colonies formed by diphtheria bacilli on solidified blood-serum or on ascitic agar are small round *raised* spots; they are variable in size, but rarely exceed that of the head of a medium sized pin. They are white or grey in colour, and opaque. They do not tend to run together so as to form a uniform film over the surface of the medium, but remain discrete even when closely packed. Some cocci form colonies which closely resemble those of diphtheria, but they rarely become elevated so high above the surface in the same space of time.

II. *Microscopical*.—Prepare films by the method described on page 29, following out all steps in the fullest detail. Stain one of them (step 11) with Löffler's blue or carbol-thionin, allowing the stain to act for two minutes, and the other by Gram's method.

In removing some of the growth to make the film, remember the facts just stated as to the characters of the colonies of the bacillus, and select a colony presenting those characters (especially that of elevation) if one is present. If there is no apparent growth in the tube take "sweeps" of the whole surface. This is conveniently done by means of a platinum loop,

shaped like a stirrup, the flat bar being drawn along the surface of the medium from bottom to top just as a rake is drawn along a flower bed.

Now examine your specimens in the way described on page 34.

#### CHARACTERS OF THE DIPHTHERIA BACILLUS.

The following are the chief points which are considered in deciding whether a given stained slide does or does not show the diphtheria bacillus.

1. The *shape* of the bacillus is very variable, and this is a feature which often affords us great assistance; a specimen in which all the bacilli present resemble each other exactly in shape and size, is not from a case of diphtheria. Diphtheria bacilli are narrow rods; they are either straight or slightly curved in an arc of a large circle or into an *f* shape (Plate I., figs. 1 and 2). Their ends are usually rounded, but it is not uncommon to find forms with one end or both sharply pointed. Lastly, clubbed forms are to be met with in almost all cultures, though they are most frequent in those which have been incubated for several days; they may be compared to a note of exclamation (!).

2. *Their size*.—Two well marked varieties occur. The long form is about as long as a tubercle bacillus (to compare it with an organism with which the practitioner may readily become acquainted) or somewhat longer; it is decidedly thicker. The short form is only about half as long and thick in proportion.

We do not know anything as to the difference in pathogenicity (if any) of the long and the short varieties of the diphtheria bacillus. They appear to "breed

true" for long periods, and cases of diphtheria caused by the one appear to have as high a mortality as those caused by the other.

Hoffman's bacillus is dealt with subsequently.

3. *Their staining reactions.*—The diphtheria bacillus stains readily with all the stains in common use for bacteriological purposes. It usually (but not invariably) stains *irregularly*, deeply stained portions alternating with others which remain colourless. This gives rise to a beaded appearance, and forms sometimes occur which can hardly be distinguished from short chains of streptococci. When a powerful stain is applied for a long time this appearance may be lost.

The diphtheria bacillus stains deeply with thionin. This often affords a certain amount of help in the diagnosis, as many other bacilli do not stain nearly so deeply in the same time.

It stains by Gram's method. A beginner should always test his results in this way. If suspicious bacilli do not retain the violet stain they are not those of diphtheria.

4. *Their arrangement.*—This is a most characteristic feature, but it is one which is difficult to describe. The old comparison to the strokes which form a Chinese letter is a fairly good one; the bacilli lie in little groups, some lying parallel to one another, and some at various angles with these. The characteristic arrangement is best seen in a specimen made from a pure culture of the short form.

Before coming to a conclusion as to the presence or absence of diphtheria bacilli from an examination of a stained film, make a very thorough search; if no bacilli are seen make several more films and examine them. When you see a group of bacilli examine it carefully

noting each characteristic and comparing it with those described above.

The beginner is strongly recommended to procure a series of slides of diphtheria bacilli from a bacteriological laboratory and to study them carefully.

Hoffman's bacillus (Plate I., fig. 2) is a modified form of the true diphtheria bacillus, and is frequently met with in throat cultures. It is about as long as the short form of diphtheria bacillus, but decidedly plumper and is more uniform in shape and size. These bacilli stain uniformly and deeply. They exhibit the same arrangement, and are often grouped in pairs. Clubbed forms do not occur.

The diphtheria bacilli which occur in films made direct from the swab are similar to those seen in cultures, but are often thicker; they may stain uniformly, and clubbed forms are rare. It is unusual to be able to make an absolute diagnosis as to their nature, but it may be done at times. Yet such an examination is often useful. If suspicious bacilli are present you should inject antitoxin forthwith; if no suspicious bacilli are seen it is safe to wait for the result of the cultural examination.

#### INTERPRETATION OF RESULTS.

*The discovery of the diphtheria bacillus in the exudate may mean:—*

- (a). That the patient is suffering from diphtheria.
- (b). That he has suffered from diphtheria and is now convalescent but is *still infectious*. The bacilli may persist for weeks or months and while they do so the



patient must be isolated and treated with antiseptic gargles.

(c). That he is in danger of acquiring diphtheria if subjected to any influence which lowers his vitality, or which would cause ordinary sore throat in any ordinary person.

(d). It *always* means that the person may communicate diphtheria to a susceptible subject.

The significance of Hoffman's bacillus is not yet settled. It is frequently found in the throat when the patient is convalescing from an attack of diphtheria, and sometimes in subjects who afterwards develop diphtheria. It also appears to cause epidemics of sore throat which do not present anything remarkable in their clinical characters. It is safest to regard any patient who presents these bacilli as being infectious. In other words, in the present state of our knowledge it is best not to draw any distinction between Hoffman's bacillus and the true diphtheria bacillus, as far as infectivity is concerned.

*A negative result may mean :—*

(a). That the patient is not suffering from diphtheria.

(b). That the swab did not touch the affected area.

We exclude errors in technique and observation.

*A sterile culture may mean :—*

(a). That an antiseptic was used too soon before taking the swab.

(b). That the diseased portion of the throat was not touched. Other parts of the mouth contain numerous bacteria, but many of them do not grow well on blood serum or ascitic agar.

We again exclude errors arising in the laboratory.

Whenever the culture tube remains sterile the examination should be repeated.

## TETANUS.

The pathology of tetanus is very much like that of diphtheria. In each disease the specific bacilli are localised at or near the region at which they enter the body, and form a toxin which is absorbed into the blood and affects distant organs. In each case research has shown that an antitoxin is formed which neutralizes this toxin and prevents it from uniting with the cells of the body, but which has not the power of turning it out from such a combination. In other words tetanus antitoxin, like that of diphtheria, is preventive but not curative. But here, unfortunately, the resemblance between the two diseases ceases. The local lesion in diphtheria is obvious and its presence causes a good deal of inconvenience to the patient; he sees a medical man early, and the diagnosis of diphtheria is made before much of the toxin has entered the blood. It is different with tetanus. In this the local symptoms are practically nil; there may be suppuration at the region of inoculation but this is so common as not to excite suspicions. The result is that the diagnosis is not made until the appearance of the symptoms referable to the nervous system indicates that the period at which antitoxin might have been used with success has gone by.

To illustrate this we will imagine that the local lesion of diphtheria to be so slight as to be unnoticed by doctor and patient. The result would be that the disease would only be diagnosed when the severe toxæmic symptoms had manifested themselves, and antitoxin would then be almost or quite useless. If it

were not for the discomfort and pain caused by the throat lesion of diphtheria the antitoxin treatment of the disease would have probably been abandoned as useless.

But tetanus may be diagnosed by means of a bacteriological examination of the local lesion before toxic symptoms have appeared, and in cases where this is done we may safely look for results from tetanus antitoxin which are as good as those obtained from the early use of diphtheria antitoxin ; for the experimental evidence in favour of the one is every whit as great as that in favour of the other.

Considerations of time would prohibit the bacteriological examination of the multitude of small wounds which are seen by the majority of medical men. But a wound which clinical experience and bacteriological research as to the occurrence of tetanus bacilli outside the body, indicates as being one which is likely to become infected with the bacillus in question, should be submitted to a careful and prolonged search for the bacillus. These are deep incised and lacerated wounds, especially those of the hand and foot, and especially if garden earth or horse-dung has been rubbed into the tissues. Wounds made with splinters should be examined, especially if there is reason to think that the splinters were dirty. The same remark applies to deep stabs with rusty nails, &c. Tetanus *may* follow a wound which heals up by first intention, but this is unlikely ; suppuration or necrosis of the edges (though not due to the tetanus bacillus itself) is present in the majority of cases.

## EXAMINATION OF PUS FROM SUSPECTED CASES OF TETANUS.

*Requisites.*—1. Slides and cover-glasses.

2. A stiff platinum loop.

3. Bunsen's burner or spirit lamp.

4. Löffler's blue or carbol thionin.

5. Materials for Gram's staining.

6. Balsam.

If cultures are to be taken add a pipette (see p. 41), a deep tube of agar to which two per cent. of grape-sugar has been added previous to sterilisation, a flask of water, and a thermometer.

*Method.*—Scrape the deeper portions of the wound with the platinum loop and spread out the secretion thus obtained on the surface of a slide. Prepare several of these slides, and fix the film by heat. Stain some by the simple stain for two minutes and others by Gram's method.

The bacillus of tetanus is about as long as the tubercle bacillus and is very slender. It stains by Gram's method. A very characteristic feature is its method of spore-formation. The spores are spherical bodies which are formed at the *extremities* of the bacilli, giving them the appearance of pins or drumsticks. The spores do not stain by the ordinary stains, and appear as colourless and highly refractile bodies (Plate II., fig. 2).

The cultures are made in agar to which 2 per cent. of grape-sugar is added, and the needle or pipette used in making the inoculation is plunged deep down into the medium. The bacillus of tetanus is an anaërobe, *i.e.*, it grows only in the absence of oxygen. The stabs are made deep in order to inoculate the material far away

from the air, and the glucose is added to absorb any oxygen which may be in the medium. To increase our chances of obtaining this bacillus in pure culture the material to be examined is to be heated to a temperature which will kill all developed bacteria, but which will not be injurious to spores; the tetanus bacillus is the only anaërobic organism with a spherical terminal spore which is at all likely to occur in a wound.

*Method.*—The inoculations are to be made with a pipette. If the pus which comes from the wound can be drawn up into the capillary tube of a glass pipette such as is described on page 41, the material should be collected in this way. If this is not the case the wound must be scraped with a sterilised platinum needle or other suitable instrument and the material thus obtained mixed with some boiled water (previously cooled) and then sucked up into the pipette; the end of the latter is then to be sealed in the flame, care being taken that the material itself is not heated.

Having filled and sealed the pipette, heat some water in a small flask or large test-tube until it reaches 80° C., as measured by the thermometer; insert the sealed end of the pipette in the water and maintain the temperature for ten minutes. The thermometer is to be kept in the water the whole of the time, and the flame is to be taken away when the temperature rises above 80° C. and reapplied when it falls below that point.

At the end of this time the pipette will contain no living object other than spores. Break off its point and insert it gently into the glucose agar, taking care to keep exactly in the axis of the tube, until the tip of the pipette reaches almost to the bottom of the test-tube. Withdraw the pipette gradually, blowing out its contents as you do so. The spores of the tetanus bacillus

(if present) will now be inoculated deep down in the medium, far away from the air. To reduce the supply of oxygen still further it is a good plan to melt some paraffin (a hard candle answers perfectly) and pour a layer an inch thick over the surface of the medium.

The cultures thus made are to be incubated for a few days at the body-temperature. After about forty-eight hours the growth begins to appear in the deeper portions of the tube as a series of delicate wavy outgrowths from the central stab. These do not appear in the upper portion of the medium, where the oxygen hinders their growth. If the tube shows such a growth it should be submitted to a microscopic examination. It is a good plan to break the tube and to split up the cylinder of medium with a knife; films are made from the growth and stained as above. Spores are formed after about thirty-six hours.

The other methods of cultural examination are far more difficult.

#### INTERPRETATION OF RESULTS.

If bacilli having the above characters are found in films, the diagnosis of tetanus must not be considered as being absolutely proven, for there are other bacilli which might be mistaken for those under discussion; but the probability that the patient will develop the disease is so strong that steps should be taken accordingly. The wound should be scraped and thoroughly treated with antiseptics, and antitoxin should be given. If the deeper portion of the glucose agar stab shows the tree-like growth which has been described and contains slender drum-stick bacilli, the case is strength-

ened, even although the upper part of the medium is contaminated with other organisms.

The only way in which the bacilli can be recognised with absolute certainty is by means of animal experiments, and to this end the practitioner should transmit to the laboratory some of the scrapings from the deeper portion of the wound in a test-tube sterilised by boiling, or, still better, by dry heat.

### THE PNEUMOCOCCUS, PNEUMONIA, ETC.

The pneumococcus is a very important organism, and one which plays a prominent part in the production of disease. It may occur in the mouth in a healthy person; hence its recognition in small quantities in the sputum is not of diagnostic value.

The pneumococcus is a very common cause of disease of the respiratory system. It causes:—

1. *Acute lobar pneumonia*. Opinions differ as to whether it is the *only* cause of this disease, though it appears most probable that this is the case.

2. *Lobular (broncho-) pneumonia*. This disease may also be caused by streptococci, staphylococci, diphtheria bacilli, influenza bacilli, plague bacilli, tubercle bacilli, and others. The pneumococcus may also occur as a *secondary infection* in lobular pneumonia due to any of these.

3. *Pleurisy*, either the serous, fibrinous, or purulent varieties. It is important to notice that the prognosis of empyema is better when the disease is due *solely* to the pneumococcus than when other organisms (streptococci, staphylococci, tubercle bacilli, &c.) are present,

and such cases usually recover without resection of ribs. The bacteriological examination of the pus from a pleural cavity may thus lead to results important as to prognosis and treatment.

4. The pneumococcus may occur as a *secondary infection* in almost any disease of the lung; for instance, in the walls of a phthisical vomica.

The most important primary lesion due to the pneumococcus outside the respiratory system are:—

1. *Otitis media*, of which it is a very common cause.

2. *Brawny induration of the skin* with or without supuration. This is uncommon, but the author has seen several cases.

Pneumococci may escape into the blood from any of these lesions, and may appear in that fluid when there is no obvious primary lesion from which it could have gained access. The most common results are:—

1. *Septicæmia*.

2. *Ulcerative endocarditis*. Many other bacteria may cause this disease.

3. *Meningitis*. Pneumococcal meningitis may also be due to direct spread from the middle ear.

4. *Arthritis*.

5. *Peritonitis*.

In actual practice we have most commonly to search for the pneumococcus in sputum, pus, and blood. In the latter case cultural methods are usually necessary, and we shall defer its consideration for the present.

*Sputum*.—The examination of sputum may be made in order to make a diagnosis as to the presence or absence of pneumonia in a case in which the physical signs are indeterminate, or to establish the nature of a lobular pneumonia.

The patient must wash out his mouth with water,



which should have been boiled and allowed to cool. He must then spit into a clean wide mouthed bottle also containing boiled water, and care must be taken that the sputum used for the examination comes directly from the lungs and is not merely mucus which has collected in the mouth.

The mass of mucus forming a single "spit" is agitated gently in the water to remove contaminations from the bronchial tubes and mouth; the water is poured off and more added, and the process repeated several times. Then the mass of mucus is fished out, placed in a watch glass, carefully opened with a pair of scissors, and a piece about as big as a pea is removed from the centre of the mass with a platinum loop. It is placed on a clean slide, another slide pressed upon it, and the two are slid apart. The films thus obtained are allowed to dry, and fixed by heat in the usual way.

One is stained in dilute carbol-fuchsin for about two minutes and then washed very thoroughly in water. The other is stained by Gram's method.

The pneumococcus is a diplococcus, *i.e.*, the individual cocci are arranged in pairs. Each coccus has usually an oval or lancet shape, the sharp ends of the two germs pointing away from one another (Plate I., fig. 3). Abnormal forms (round cocci, short bacilli, &c.) are frequent. The pneumococcus has a *capsule* when it occurs in the living body or in pathological exudates, but not in most cultures. This capsule does not stain readily, and appears in a properly stained specimen as a clear halo round the two cocci.

Examine your Gram specimen first. The pneumococci should be clearly seen, and you should be able to make out their shape and characteristic arrangement in

pairs. The rest of the slide will be unstained, so that you will not be able to make out the capsule.

Now examine the carbol-fuchsin specimen. You will see the cocci coloured a deep red, and you will also notice that the general surface of the film is stained pink, while there is a clear and colourless zone round each pair of cocci. This is the capsule, which is rendered distinct by "negative staining." If the carbol-fuchsin has been allowed to act too long the capsule may be stained a faint pink.

#### INTERPRETATION OF RESULTS.

In cases of lobar pneumonia you will probably find pneumococci in great quantity, and no other bacteria in a specimen of sputum *made in the manner described*. If you find many pneumococci in a case of lobular pneumonia the disease may have been caused by another germ, and the cocci in question may have been nothing more than a secondary infection. The carbol-fuchsin specimen should be searched for bacilli resembling those of influenza, &c., and another should be stained for the tubercle bacillus if the clinical aspect of the case suggests the possibility of a tubercular origin for the disease.

*Pus* is examined in the same way and presents similar appearances. Most of the cocci are extra-cellular.

The method of making a film from pus is described in a subsequent chapter. It is very similar to that described above, but the upper slide should be allowed to fall on the lower one by its own weight; the two should not be pressed together.

## INFLUENZA.

The diagnosis of influenza is often very difficult, and a simple bacteriological examination should be made in all cases. The cultivation of the specific bacillus is by no means easy, but this does not matter, as an examination of stained films will usually permit of a diagnosis.

The influenza bacillus occurs in the sputum and occasionally in the blood. It may be searched for in the latter situation, but the quest is a very difficult one. In the sputum it occurs in vast quantities and is almost free from other germs.

The method by which the sputum is collected is the same as that employed in pneumonia; a mass of greenish-yellow muco-pus is selected for examination, squeezed between two slides, and the films dried and fixed as in the case for the examination for the tubercle bacillus. One is stained for five minutes with dilute carbol-fuchsin or Löffler's blue, the slide being slightly warmed, and the other by Gram's method.

## EXAMINATION OF THE SPECIMENS.

The specimen stained with carbol-fuchsin or Löffler's blue should be taken first and examined under the oil immersion lens. The bacilli (in a positive case) will be seen in vast numbers as extremely minute rods; it would take from twelve to sixteen of these rods to make up the diameter of a red blood corpuscle. They frequently occur within the pus cells, and when in this

situation they may appear to have a capsule and present a resemblance to pneumococci, from which, however, they may be distinguished by their small size, and by the fact that they do not stain by Gram's method (Plate II., fig. 3).

Now examine the Gram specimen. The bacilli are not seen, though other bacteria (pneumococci, streptococci, staphylococci, &c.) may be visible.

#### INTERPRETATION OF RESULTS.

If minute bacilli having the above characters are found in large numbers in sputum taken in the manner described, the diagnosis of influenza may be made with certainty.

If none are apparent and you are sure of your technique (and also of the fact that the sputum came from the lungs) the diagnosis of influenza is highly improbable.

If the pulmonary symptoms in a case of influenza do not clear up speedily the sputum should be examined for the tubercle bacillus and the examination repeated at intervals.

#### ANTHRAX.

Anthrax occurs in man in three forms. The most common is *cutaneous anthrax*, or, as it is sometimes called, malignant pustule. *Pulmonary anthrax* or wool-sorter's disease is much rarer, and *intestinal anthrax* rarer still. The practitioner will find the greatest

assistance from a bacteriological examination in the cutaneous form of the disease; he may search for the specific bacillus in the sputum in a supposed case of wool-sorter's disease, but he must be careful in his interpretation of his result, as bacilli which might be mistaken by an untrained observer relying on the morphological appearances alone, sometimes occur in the sputum. The search for bacilli in the fæces in a supposed case of intestinal anthrax must be relegated to an expert.

The true nature of a case which is examined post-mortem can easily be determined bacteriologically; the cut surface of the liver or spleen should be rubbed upon a clean slide, and the films treated *secundum artem*. They will probably show the bacilli in large numbers. Sections may also be cut, or portions of the organs fixed in the manner to be described subsequently and forwarded to a bacteriological laboratory.

In the later stages of any infection with anthrax the bacilli may be found in the blood. They may be apparent on examination of stained films, or by cultural methods similar to those used in the diagnosis of malignant pustule.

The anthrax bacillus varies considerably in length, but is always a large organism and may be considerably longer than the diameter of a red blood corpuscle. It is much thicker than the bacilli which have been dealt with hitherto, and it is invariably straight. The ends of these bacilli are cut sharply at right angles to the sides of the organism, and may be even somewhat concave; this is a most characteristic feature. The anthrax bacillus stains by Gram's method (Plate I., fig. 4).

In cultures the appearances are somewhat different.

Here the bacilli are frequently arranged in long chains which have an appearance which has been compared to that of a bamboo; chains occur in the blood or in the inflammatory exudate, but are usually much shorter than those seen in cultures. But the most important feature in cultivation of the anthrax bacillus is the development of *spores* which are oval highly refractile bodies, which lie in or near the centre of the bacilli, one in each. These spores are possessed of tough capsules, which resist the action of the ordinary stains much in the same way as the tubercle bacillus does. Thus it happens that in films of a cultivation of the anthrax bacillus which have been stained with such a dye as methylene blue the spores are readily seen as colourless and refractile oval areas in the centre of the bacilli, the latter being stained blue. The spores themselves may be stained by a modification of the process used for the tubercle bacillus. The films are first stained by heated carbol-fuchsin, which penetrates slowly through the capsule; they are then decolorised by a *very rapid* immersion in dilute sulphuric acid and examined microscopically. If the red colour has been entirely removed by the spirit they are ready to be counterstained by methylene blue; if not they must be dipped in the spirit once more and re-examined. When this process is successful the spores are stained red and the bacilli blue.

The presence of spores enables us to isolate the bacilli from most of the organisms with which it is likely to be contaminated by a very simple process. The spores resist the action of heat just as they resist stains, and for the same reason, and suitable temperature will kill off all the non-sporing organisms and spare the spores. The latter may then be inoculated at a suitable tem-

perature and will develop into bacilli. This process, however, is not applicable to the examination of the blood or morbid effusions, as the bacillus of anthrax does not form spores in the living body. In this it differs from the tetanus bacillus.

#### INVESTIGATION OF A SUPPOSED CASE OF MALIGNANT PUSTULE.

*Requisites.*—1. Several glass pipettes; if cultures are not required one will be enough.

2. Clean slides and cover-glasses.

3. Bunsen's burner or spirit lamp.

4. Löffler's methylene blue; also the materials for Gram's staining.

5. Balsam.

6. Tubes of gelatin if cultivations have to be taken.

*Method.*—Break off the extreme tip of one of the glass pipettes and insert into one of the vesicles around the dark papule in the centre of the lesion; it may be necessary to make a puncture with a sterilised needle before this can be done. If the fluid does not rise spontaneously into the pipette break off the other end and suck gently, watching the column of fluid so that it does not get into your mouth.

Having obtained a drop or two of the fluid exudate blow it out on to the surface of a clean slide and spread it out into a film; prepare as many of these as you can. Allow them to dry, and stain one with Löffler's blue, and some by Gram's method.

Examine with the oil-immersion lens. Make a careful search over the films, looking for large cigarette-shaped bacilli, noting whether they are or are not

arranged in chains. Examine the Gram specimens and see whether the bacilli are to be seen in them also.

#### INTERPRETATION OF RESULTS.

If the case is really one of malignant pustule the chances are very greatly in favour of your finding the bacilli in large numbers, and the failure to do so tells strongly against a positive diagnosis.

*Cultural methods.*—The fluid for examination is taken in exactly the same way as that described above, but the isolation of the organisms will be greatly facilitated if antiseptic methods are employed to prevent contamination with skin bacteria. To this end the surface of the lesion should be washed gently with carbolic or perchloride lotion and then (very thoroughly) with alcohol or methylated spirit to remove the antiseptic. The surface is then allowed to dry.

If the material is to be transmitted to a public laboratory for examination (and this is the wisest course to adopt) the fluid must be carefully sucked up into the bulb and both ends of the pipette carefully sealed.

If the examination is to be made at home the best way is to make two inoculations in gelatine. The first should be a stab culture and may be made with the pipette direct; or the fluid may be blown out into a watch-glass or on to the surface of a slide (in either case sterilised by being heated in the flame and then allowed to cool) and the stab made by dipping the end of a straight platinum needle into the fluid and then driving it into the gelatin.

The other culture is made with the pipette; this is driven into the gelatin in a tube and the contents blown



out. The gelatin is then immersed in *warm* water until it is melted and poured out into a Petri's dish



FIG. 13.—Petri's Dish.

(fig. 13) previously sterilised by dry heat and allowed to cool.

Both cultures are incubated at a temperature of about 20° C.



FIG. 14.—Stab culture of anthrax bacillus (Crookshank).

In about two days the gelatin stab tube will show a very characteristic appearance if the anthrax bacilli are

present in pure culture. The growth takes place in lines which project nearly at right angles to the line of inoculation, and grow more vigorously the nearer they are to the surface. The result is the development of a culture which has a strong resemblance to an inverted fir-tree (fig. 14). In another day or two the gelatin will begin to show a certain amount of liquefaction at the surface.

The appearances in the plate-culture are perhaps not quite so characteristic, but they are manifested in impure cultures. The young colonies of anthrax bacilli



FIG. 15.—Young colony of anthrax bacillus ( $\times 15$ ). (Crookshank).

have a whorled appearance which has been compared to a barrister's wig or to the head of Medusa (fig. 15). The plate should be placed upon the stage of the microscope and examined for these colonies with the low power. If one is found a clean cover-glass should be pressed upon it, lifted up with a needle so as to bring up the colony with it, fixed by heat, and stained with carbol-thionin or methylene blue. The colonies are most characteristic after two days incubation; at a later period the gelatin is liquefied and spores are formed.

A culture which presents these cultural and morphological appearances may be considered to be one of anthrax with almost absolute certainty, though other tests (notably animal tests) would be applied in a laboratory.

## TUBERCLE.

The diagnosis of tuberculosis by bacteriological methods (in the case of most morbid exudates) is within the reach of every practitioner; cultural methods are not used and the recognition of the bacillus is rapid, easy, and certain.

The bacilli may be sought for in sputum, urine, pus, fæces, or any other morbid material. We will first describe the method of staining which should be adopted, then the characters on which the recognition of the bacillus depends, and lastly the methods by which the films are prepared from the various materials.

### STAINING THE TUBERCLE BACILLUS.

*Requisites.*—1. Slides, cover-glasses, forceps, and balsam.

2. A Bunsen burner or spirit lamp.

3. Dilute sulphuric acid—about 20 per cent.—contained in a wide-mouthed bottle or in a jar. This must be large enough to admit a slide but not large enough to permit it to fall down to the bottom.

4. Carbol-fuchsin.

5. Methylene blue. (Saturated watery solution or Löffler's blue).

6. A metal (iron or copper) plate. The exact dimensions do not matter, but  $8 \times 4 \times \frac{1}{8}$  in. is a convenient size. It should be mounted upon a tripod. This slab is not absolutely necessary, but it is a very great advantage.

*Method.*—We will suppose that the film has been prepared by one of the methods described subsequently and fixed by heat.

1. Place it upon the metal plate and heat the latter by the flame. Flood the slide with carbol-fuchsin and let the heat continue until the stain steams, but do not allow it to dry up; let this go on for from three to five minutes. If the stain shows signs of drying up add a little more; if it begins to boil slide it along the plate away from the flame, or remove the latter for a short time.

If you have no metal plate it is possible to hold the slide with a pair of forceps, but in this case the film is most conveniently made on a cover-glass.

Remember not to let the stain dry up.

2. Remove the slide from the plate with the forceps and wash it under the tap or in a bowl of water.

3. Put it into the bottle containing the dilute acid. After three or four minutes withdraw it and again wash. If much pink colour comes back re-insert it in the acid for a short time and again wash. The process must be repeated until the film only shows a slight pink tinge.

4. Now apply the methylene blue for a minute or so.

5. Wash, dry with blotting paper and then by gentle heat. Apply a drop of balsam and cover.

## RECOGNITION OF THE TUBERCLE BACILLUS.

The tubercle bacillus is about half as long as a red blood corpuscle is wide, or rather longer, and is very slender. It is straight or slightly curved, and is variable both in shape and in size (Plate II., fig. 2).

We recognise it by means of a staining reaction. Tubercle bacilli contain a considerable amount of fat, and this prevents them from staining readily with ordinary stains. In the process described above we used fuchsin, which is a very powerful stain, and added a mordant (carbolic acid) which increases its penetrative properties. Even with this, staining is very slow, so that we heated the specimen.

The fat which prevents the bacilli from staining also prevents the stain from being removed by such substances as acids and alcohol. In stage 3 of the above process we aim at allowing the acid to act until it has removed the fuchsin from everything except the tubercle bacilli. The methylene blue is a counter-stain, and colours all organisms, pus cells (especially their nuclei), epithelial cells, and shreds of lung tissue; in fact everything except the tubercle bacilli. The latter appear as slender red rods which often show the irregular staining which has been described as occurring in the diphtheria bacillus.

Now "acid-fast" bacilli are very rare, though they have been found in unexpected situations of late years. Only three such bacilli need be taken into consideration in dealing with human pathology. These are the tubercle bacillus, the leprosy bacillus, and the smegma bacillus. The bacillus of leprosy would rarely lead to

mistakes in this country; it is recognised by the fact that it is straighter and more uniform than the tubercle bacillus, and by the fact that it resists decolorisation more powerfully than the tubercle bacillus. The smegma bacillus may occur in the urine and lead to mistakes unless the sample examined was drawn off per catheter. It is distinguished by the fact that it is readily decolorised by alcohol (absolute alcohol or methylated spirit) while the tubercle bacillus is not. In staining a film from the urine we decolorise in spirit for a minute after the acid and before the methylene blue, *i.e.*, between stages 3 and 4 in the above description.

In searching for the tubercle bacillus the  $\frac{1}{8}$  in. lens will serve, though an oil immersion lens is an advantage.

#### METHOD OF COLLECTING THE SPUTUM.

This is of some importance, and the method recommended should be carried out in all cases.

Get the patient to wash out his mouth thoroughly with warm water before going to bed. Let him spit into a clean bottle, jar, or tin, and employ only the sputum coughed up before food is taken in the morning.

#### METHOD OF PREPARATION OF THE FILM.

*Sputum.*—Pour the sputum into a watch-glass and place the latter upon a dark surface. Examine it closely, looking out for small yellow particles; these consist of caseous material and will probably contain

tubercle bacilli in large numbers. The advantage of getting the patient to wash out his mouth and using only fasting sputum is obvious, for particles of food may present exactly the same appearance.

Having found such a mass pick it out by means of a platinum loop or pair of forceps and transfer it to the middle of a clean slide. Now place another slide on the top of the first, squeeze them together and then slide them apart. You should get two good uniform films. Dry and fix.

If there are no caseous masses pick out a mass of the sputum at random and proceed as before.

A better method is as follows:—Half fill an ordinary medicine bottle with carbolic lotion (1 in 20) and add a drachm or two of the sputum. Shake thoroughly for a few minutes and place the bottle where you can give it an occasional shake during the next few hours. Then pour the milky emulsion which results into a conical urine glass and allow it to stand for twelve hours or more. Remove some of the deposit which will form with a pipette and spread it into a thin film on a slide. Dry and fix.

*Urine* is treated in a similar way; but here carbolic acid (liquefied or in crystals) is added to the urine in amount sufficient to convert it into a 1 in 20 solution. This is allowed to deposit or (better) is centrifugalised. Films are prepared from the deposit.

Remember that they should be passed through alcohol after staining.

*Pus* is best carbolised in the same way as sputum; if very thin it may be treated like urine. The tubercle bacilli will rarely be found in pus unless it is examined soon after the abscess is opened, but may be detected by inoculation experiments for long periods.

*Clear exudates* are more difficult to examine, and, as they usually contain bacilli in very small numbers only, a negative result should not be given too much weight. They may be allowed to sediment in a conical urine glass, a few crystals of camphor being added to prevent bacterial growth, carbolic acid being inadmissible as it precipitates the proteids. The examination is best made in a bacteriological laboratory, as decisive results can only be obtained by animal experiments. Collect the fluid in a bottle which has previously been boiled in water for half an hour and allowed to cool. Cork it with a cork which has also been boiled. Add no antiseptic and forward it to the laboratory as soon as possible.

*Milk* may be examined in the same way as urine, films being made from the cream as well as from the deposit. These films are fixed, soaked in ether to remove fat, and again fixed. They are then stained as before, and it is advisable to pass them through alcohol.

When *faces* are to be examined the best plan is to administer opium in amount sufficient to cause constipation. The surface of the scybalous motions which result are to be scraped off and stained in the usual way.

#### INTERPRETATION OF RESULTS.

The finding of tubercle bacilli in the sputum is conclusive evidence of tuberculosis of the lungs, but no information as to prognosis can be drawn from the numbers which are present; they may occur in great quantities in the sputum from patients who are doing well, and the author has found enormous numbers in



the sputum of a person who had presented no symptoms of the disease for eight years and was apparently cured. But a person in whom the bacilli are present is always in danger of a recrudescence of the disease, *and may be a source of infection*. Absence of the bacilli does not disprove the diagnosis of tuberculosis; bacilli do not appear in the sputum until the lung-tissue in which they occur breaks down, and are therefore absent in the early stages of acute tuberculosis.

In some cases of ordinary chronic phthisis bacilli may occur in the sputum in very scanty numbers and may be missed unless a very careful search is made. Bacilli should not be considered as being absent until well stained films have been examined for *at least* half an hour, and the examination repeated on several occasions.

The finding of tubercle bacilli in the urine is practically absolute proof of tuberculosis of some part of the urinary tract, probably the kidneys or bladder. Absence of bacilli implies nothing unless the examination has been made very thoroughly and repeated several times at intervals. Then it affords *presumptive* evidence that the urinary passages are free from the disease.

The same is true of the examination of pus. Tubercle bacilli rarely occur in inflammatory exudates except in very small numbers and can only be demonstrated by animal experiments. If you examine pus from a chronic abscess and find no organisms of any kind it is almost certain that the process is a tubercular one; and the negative evidence obtained by the failure to find tubercle bacilli should not be allowed to carry much weight. The same is true for the clear exudates.

## LEPROSY.

The leprosy bacillus resembles that of tubercle, but it is somewhat straighter and more uniform. It occurs in leprous lesions in great profusion, and its discovery does not present any difficulty. It has never been cultivated.

In a suspected case of leprosy, films should be made from the nasal discharge, for the nasal cavities are very frequently affected. Indeed, it seems highly probable that the primary lesion through which the bacilli gain access to the body is in the nose in most cases. A small portion of one of the leprous nodules may also be removed and films made by rubbing the cut surface against a clean slide. If there is an ulcer films may be made from the secretion from it.

Films should be stained by the method which we have recommended for the tubercle bacillus. If bacilli are present in large quantities the case is almost certainly one of leprosy, for tubercle bacilli are never found in similar situations except in scanty numbers. If a doubt should arise as to the identity of the bacilli, advantage should be taken of the fact that the leprosy bacillus retains the fuchsin even more firmly than the tubercle bacillus when exposed to the action of an acid. A film from the suspected material should be spread at one end of a slide, and some sputum known to be rich in tubercle bacilli at the other; the whole should be stained by hot carbol-fuchsin and decolorised by being immersed bodily in 25 per cent. sulphuric acid for half an hour. If the tubercle bacilli are decolorised any bacilli which have retained the red colour are almost certainly those of leprosy. If the tubercle bacilli are

not decolorised a fresh specimen should be prepared and immersed in the acid for a longer period.

## ACTINOMYCOSIS.

Actinomycosis is very closely allied to tuberculosis; the lesions appropriate to the two diseases are almost identical in histological appearance, and the granuloma which occurs in actinomycosis goes on to fibrosis or to the formation of "cold abscesses" just as a tubercle may do. The formation of fibrous tissue is most marked in cattle, and in them the disease is more chronic; suppuration is more common in man, and the disease runs a more rapid course.

The pus from an actinomycotic abscess is often viscid and contains a greater or smaller number of small greenish, yellow, or brownish nodules. They are about as large as the head of a very small pin, and are quite opaque; under the low power of the microscope such a granule has a coarsely granular appearance, and looks something like a raspberry. If nodules presenting these appearances are found in any specimen of pus, whatever be its origin, a careful microscopic examination should be made to determine its nature. This is not difficult.

*Method.*—Place some of the pus which contains these granules on a clean slide and press another slide upon it so as to crush the granules, dry, fix, and stain by Gram's method.

Tumours removed or incised at an operation, or organs removed at a post-mortem examination, should have their cut surfaces rubbed upon the surface of a slide and the film thus obtained treated in a similar

way ; or they may be scraped, and the scrapings spread on a slide. Sections may also be cut, but are not usually necessary for the diagnosis.

#### EXAMINATION OF THE SPECIMENS.

Actinomycosis is caused by the ray-fungus, an organism which derives its name from the star-shaped colonies which it forms whilst growing in the tissues. It consists of two chief parts ; the central portion of the colony is formed of a network of narrow filaments, which have a radial arrangement at the periphery (Plate II., fig. 6). In this part small bodies which have the appearance of cocci may often be seen. The outer zone consists of the clubs which (when present) are so characteristic. These clubs are flask-shaped expansions of the sheath of the radial filaments already mentioned, and are arranged with their narrow extremities pointing inwards. They are not generally present in man, and when present are often badly developed ; they are much more common and more perfect in the ox, where the disease is more chronic, and pus formation rare.

The films should be carefully examined for the presence of these structures. Clubs are not likely to be found in the pus, and their absence does not tell against the diagnosis ; the dense felted network of filaments retaining Gram's stain is what is to be looked for, and its presence is quite sufficient for a diagnosis. Fortunate specimens may show a complete colony, with its irregular network in the centre and the radial arrangement of the fibres on the periphery, or there may be mere fragments of mycelium.

## GLANDERS.

Glanders is one of the infective granulomata and is closely allied to tuberculosis: it differs, however, in running a more rapid course and in the greater tendency which the specific lesions exhibit to undergo suppuration. It is caused by the bacillus mallei, an organism which is nearly as long as the tubercle bacillus and decidedly thicker. It stains readily with all stains and is easily decolorised; it loses its stain when treated by Gram's method, and does not form spores.

The bacteriological diagnosis of the disease is not easy, and should be referred to a public laboratory. A quantity of the discharge from a suspected case should be taken with aseptic precautions and transmitted as soon as possible in a test tube or bottle which has been sterilised by dry heat or by boiling. Pus had better be sent in a pipette.

Where abscesses are opened cultures taken direct from the pus may possibly contain the bacillus in pure culture. In this case it may be identified by the characters of its growth upon potato. The colonies have the colour and appearance of honey at first; they grow very rapidly, coalesce, and the potato is soon covered with a moist-looking film which afterwards becomes brown, the surface of the medium in the neighbourhood becoming greenish-brown. If cultures from pus grown on potato exhibit these appearances and contain a short and thick bacillus which does not stain by Gram's method, the case may be diagnosed as being probably one of glanders, even although the culture be not a pure one.

The injection of mallein would probably supply information which would be obtained more easily and quickly, and would be more conclusive.

## TYPHOID FEVER.

Typhoid fever is caused by a bacillus which is variable in length, though usually short (about half as long as a tubercle bacillus) and thick, its length being only about three times its breadth. It does not form spores, and it does not stain by Gram's method. It is actively motile; when a culture of the organism in a fluid medium is examined under the microscope the bacilli can be seen darting rapidly about in all directions. It owes its motility to the possession of a large number of long wavy flagella, which can only be seen after special and difficult staining processes.

The bacillus coli communis, the most plentiful organism of the intestine in man and animals, bears a very close resemblance to the typhoid bacillus, and can only be distinguished therefrom by the application of several cultural and chemical tests, the performance of which takes a considerable amount of time. This renders it very difficult to diagnose typhoid fever by methods similar to those which are in use for the other diseases mentioned, *i.e.*, by the demonstration of the specific organism. Suppose for instance, that we were to attempt to determine the nature of a case of diarrhoea by a search for the typhoid bacillus in the stools. For every typhoid bacillus which we should encounter we should find a great many colon bacilli, and we should only be able to distinguish the one from the other by

a prolonged and careful examination of pure cultures. It is quite certain that the disease *might* be diagnosed in this way; indeed, it has been done, but the task is an extremely difficult one and the diagnosis would be delayed for a considerable period.

In other regions in which the typhoid bacillus occurs during an attack of typhoid fever the search is usually facilitated by the absence of other organisms, especially by the absence of the bacillus coli. The specific bacillus occurs in the blood, spleen, spots, mesenteric glands, liver, and frequently in the urine.

It may often be demonstrated in the blood, but a large quantity must be taken, and the technique is difficult. The results obtained in this way are of great scientific interest, but the process is of no value as a means of diagnosis. The same remark applies to the spots; it is in fact somewhat doubtful whether the bacilli which appear to be present in this situation do not actually come from the blood which is drawn in the process.

The mesenteric glands and liver are of course not available for the purposes of diagnosis.

The demonstration of the bacilli in the urine is sometimes quite easy, and might be of some diagnostic value. But they do not always occur in this excretion; and the bacillus coli may do so.

The only way in which typhoid fever can be diagnosed with ease and certainty by a demonstration of the specific bacillus is by an examination of material drawn directly from the spleen by means of a hypodermic needle. The organism occurs *constantly* in this situation, and its demonstration is not difficult. The necessary operation, however, is by no means devoid of risk, and is now generally abandoned.

This brings us to the only method in which typhoid fever is now diagnosed by the bacteriologist—the Widal's reaction. This reaction is a special example of a general law which was discovered by Durham and others, and which is to the effect that the blood serum of a person who has been through an attack of a bacterial disease will cause the specific organism of that disease to collect into clumps. For instance, if we take a broth culture of the vibrio of Asiatic cholera (which is turbid and opalescent) and add to it a small quantity of blood-serum from a patient who has recovered from an attack of cholera we shall find that the culture becomes clear, a sediment collecting at the bottom of the tube; and if we examine this sediment we shall find that it consists of felted masses of the vibrios. This reaction is a general one, and is given in most, if not all, bacterial diseases. But Widal, Grünbaum, and others, working independently about the same time, showed that whereas in many diseases it is a reaction of *immunity* (i.e., does not occur until late in or after the disease) in typhoid fever it is a reaction of *infection*, and occurs so early in the course of the disease that it is of great value in diagnosis.

The test is applied by adding a small quantity of the serum from the patient suspected to be suffering from the disease to a larger amount of a young culture of typhoid bacilli, and watching whether the appearance of the culture undergoes any change: it may be watched under the microscope or by the naked eye, the technique differing in the two cases. The *microscopic method* is rapid and requires a very small amount of blood, and is now generally used. The *macroscopic method* is perhaps somewhat easier for a beginner, but it takes a longer time and requires a larger amount of blood serum.



The technique in either case is readily learnt and can be carried out with very little difficulty if the materials are at hand. But the test is one which is seldom advisable for the home worker to attempt, as it requires a young culture of typhoid bacilli. We shall, however, describe the process, as some may carry it out during an epidemic, or if they are living where they have not access to a public laboratory. The process has now been greatly facilitated by the demonstration of the fact (by Widal) that the reaction is given with dead cultures of the bacilli. These cultures can be obtained from any bacteriological laboratory, and will keep. They are prepared by adding four or five cubic centimetres of normal saline solution to an eighteen-hours-old culture of the typhoid bacillus on agar, scraping off the growth, filtering to remove clumps, and heating the emulsion to 60° C. for ten minutes to kill the bacilli. A small quantity of carbolic acid should be added to prevent putrefaction. With a supply of this culture on hand the practitioner can apply the test at home without much difficulty.

Where the blood is to be transmitted to a laboratory it should be sent in a pipette specially made for the purpose. This consists of a bulb terminating in a capillary tube at one end and in a mouth-piece (which is plugged with cotton-wool) at the other (fig. 12, c). To procure the blood wash the patient's skin and make a deep puncture with a sterilised needle; further antiseptic precautions are unnecessary. When a large drop of blood has collected upon the skin (and this may be hastened by gently squeezing the region, or by allowing the finger to hang down) the tip of the pipette is inserted and suction applied. The practitioner must aim at filling the *whole* of the bulb with blood, and must

avoid air-bubbles. This is because the test is easier to carry out and is more conclusive if clear serum can be obtained. The test may be applied to a dry drop of blood, but in this case there is no possibility of making an accurate dilution. The test is wonderful enough as it is, but the practitioner should not expect too much from it if he sends a few splashes of blood inside a capillary tube of the pipette. This is so important that we repeat: do not be satisfied unless the bulb is completely filled with blood. When this is accomplished suck the fluid a little further up and seal the pointed end of the pipette in a flame—the flame of a wax match will answer.

If a pipette is not at hand it is advisable to manufacture one out of any piece of glass tubing which may be available. Failing this the best plan is to dry several large drops of blood on a clean slide; but results obtained from blood forwarded to a laboratory in this way should be received with caution, for the dilution is necessarily a matter of guess-work.

Do *not* send blood in vaccine tubes. When this is done the blood often adheres so firmly to the glass that the whole tube has to be ground down in a mortar with a drop or two of water.

#### METHOD OF PERFORMING WIDAL'S REACTION BY THE ' MICROSCOPIC METHOD.

*Requisites.*—I. A young culture (not more than eighteen hours old) of typhoid bacilli on agar.

Where dead bacilli are to be used this is to be replaced by a culture obtained from the laboratory and prepared in the manner described.

2. A small funnel provided with a double thickness of white filter paper. This is unnecessary if dead cultures are to be used.

3. Three clean watch-glasses.

4. A platinum loop. This should be made of fine wire and have a loop (which must be completely closed) about  $\frac{1}{16}$  in. in diameter.

5. A hollow-ground slide. This is an ordinary slide having a well about half an inch in diameter sunk in its centre. If it is not at hand a cell may be built up on an ordinary slide. Take a piece of thin card one inch square and cut out a square half an inch in diameter from its centre. Fix this perforated square down on to the slide with vaseline or immersion oil.

6. Thin cover-glasses.

7. The microscope. The test can be carried out quite well with a  $\frac{1}{4}$  in. lens.

*Process.*—1. *Making the emulsion.*—Pour a small quantity of tap water into the culture tube, or better, scrape off some of the growth and mix it with some water in a watch-glass. In either case stir it round with the platinum needle for a few minutes so that the bacilli are evenly distributed throughout the water and form an emulsion.

Next take the hollow-ground slide and paint a ring of immersion oil round the well (fig. 16, *b*). If you are using a built-up cell paint the top of the card with the oil. In either case vaseline may be used.

Place a drop of the emulsion on a clean dry cover-glass and invert the hollow ground slide over it; press it down so that the oil round the well adheres to the cover-glass; now invert the slide and you will have a *hanging-drop* specimen. The bacilli will be contained in the droplet of water (fig. 16, *a*) which hangs from the

lower surface of the cover-glass ; this will not dry up if the seal made by the oil is perfect.

Place the specimen under the microscope and examine it with the low power, using the fine adjustment and stopping down the diaphragm. Focus until the surface of the cover-glass is distinctly seen, and then move the slide about until the edge of the hanging-drop runs across the centre of the field. Then turn on the  $\frac{1}{8}$  in. lens and open and shut the diaphragm until the field is faintly illuminated ; the exact amount of light required can only be learnt by experience.

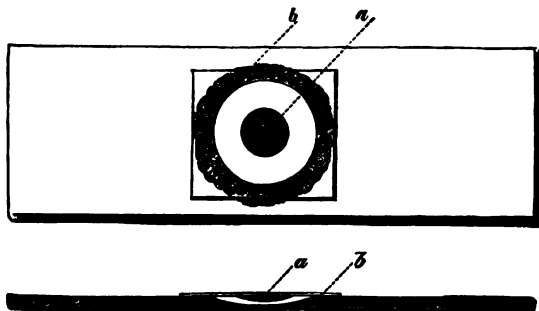


FIG. 16.—Hanging-drop preparation (Crookshank).

Now focus up and down *very carefully*, using the fine adjustment, until you see a line running across the field and dividing it into a lighter and a slightly darker portion. This is the edge of the drop. Focus a little deeper ; you should see numerous small unstained bacilli, and if these are not visible it probably indicates that the illumination is not right. Open and shut the diaphragm, keeping a sharp look out down the microscope all the time. It may help matters to lower the condenser for a short distance.

Having obtained a clear view of the bacilli examine them for motility and absence of clumps, and see whether they are present in proper proportion to the amount of fluid.

If the culture is in good condition the bacilli should be seen darting about in all directions, but if the movement is but sluggish the reaction may still be obtained. If the specimen is kept for a short time in a warm place or in the incubator the movements will usually become more rapid. It is hardly necessary to say that when dead cultures are used there will be no movements of translation, though the bacilli may show oscillatory (Brownian) movements.

The specimen must be searched thoroughly for clumps of bacilli, and if these are present the emulsion must be filtered through a double thickness of white filter paper. This examination for clumps is a most important part of the process, and must be attended to whether dead or living cultures are in use.

Next see that the emulsion is neither too thin nor too thick. No definite rules can be given, but if there are very few bacilli in the field a further supply of growth must be added to the stock of emulsion, and a further specimen examined. If the bacilli are thickly crowded together the emulsion must be diluted with a little water and re-examined.

When you are satisfied that the emulsion is right, slide off the cover-glass and drop it into some antiseptic lotion; of course this is unnecessary if dead cultures are used.

2. *Making the dilution.*—You are now about to dilute a drop of the serum from the patient with a known multiple (in this case thirty times) of its bulk of the emulsion which you have just prepared. To do so you

will take advantage of the fact that the platinum loop if dipped into a fluid and pressed against a surface so that every part of the loop touches that surface will deposit a drop of fluid of definite size. You are about to mix one loopful of the serum with twenty-nine loopfuls of the emulsion just prepared and examined.

Blow the blood from the pipette out on to a watch-glass (to do this it will be necessary to break the tip of the pipette) and tilt the latter so that the serum flows away from the coagulum. Now take a loopful of the serum and place it on another watch-glass, taking care to put the loop flat on the surface of the glass; this is done more easily if the wire is slightly bent, or if a flat slide is used instead of the watch-glass.

Next heat the platinum loop in the flame; this is to burn off any blood which might remain on it and contaminate the emulsion. Take up a loopful of the emulsion and place it on the watch-glass by the side of the drop of serum, but not touching it. Repeat this until you have placed twenty-nine drops of emulsion round the serum. Mix the whole together by stirring them thoroughly with the platinum loop, place a droplet of the mixture on a clean cover-glass, and make a hanging-drop specimen and examine as before.

If the blood comes from a case of typhoid fever (with certain restrictions which will be discussed below) the microscopic appearances will be quite different from those seen in the drop of emulsion which was previously examined. The bacilli will no longer swim about rapidly in all directions; they will become paralysed, and remain quite motionless. Further, they will collect into clumps; each clump consisting of a larger or smaller number of bacilli arranged in a felted network resembling that seen in a heap of "spellicans"

(fig. 17, *b*). This is the complete positive reaction; it consists of two parts, clumping and paralysis, and is given *only* (in the dilution used) by the blood of a patient who is suffering or who has suffered from typhoid fever. If this is not the case the bacilli will continue to move about just as before, and will not collect into clumps.

In the process which has been described above, the blood has been diluted to thirty times its volume, and this is the best dilution to use for diagnostic purposes. But the reaction is given earlier if a lesser dilution (one

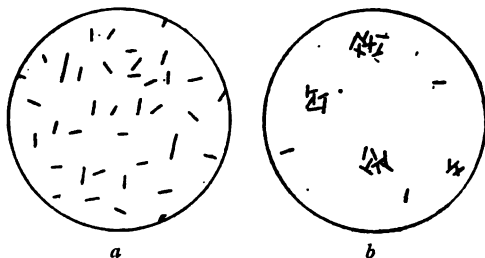


FIG. 17.

*a.* Negative Widal's reaction.      *b.* Positive Widal's reaction.

in ten) is used, though there is then a greater chance of fallacy.

Sometimes the reaction takes place almost as soon as the serum is added. At other times it is delayed, and for these it is necessary to fix a time limit. With a dilution of one in thirty, one hour is a safe time limit to adopt, and if the reaction takes place after this the result should be looked upon with great suspicion, and the test reapplied after a day or two.

Exactly similar processes are carried out if dead cultures of typhoid bacilli are used, but here the emulsion

should be sent out from the laboratory free from clumps, and containing exactly the right number of bacilli, so as to be ready for immediate use. But the practitioner is urged not to trust to such an emulsion without making a hanging-drop, and examining it just before making the test.

If dead cultures are used it is advisable to use a rather less degree of dilution than in the above process. A dilution of one in twenty will answer perfectly. The time limit is the same.

#### INTERPRETATION OF RESULTS.

A *positive* result may mean :—1. That the patient is suffering from typhoid fever.

2. That he has suffered from typhoid fever within a certain period before the blood was taken. The hypothetical substance which we believe to be the cause of the reaction (agglutinin) continues to be formed or remains in the blood for some time after complete convalescence from typhoid fever ; the reaction has been known to persist for seven or eight years, and probably usually does so for about two. This fact must be remembered in interpreting the results obtained from Widal's test. If the patient has suffered from typhoid fever, or from an obscure illness which might possibly have been typhoid fever, a year or two previously, the positive reaction should be regarded with suspicion.

In such cases the test should be carried out so that the smallest dilution which will cause clumping can be ascertained, and the test repeated in two or three days. If, for instance, we found that the blood clumps only in a dilution of one in twenty on one day and in a dilution



of one in a hundred three days later, this affords a certain proof that the reaction is due to a present attack of typhoid fever, and is not due to one which took place at a previous date. This investigation should be entrusted to an expert bacteriologist and plenty of blood sent on each occasion.

A *negative* result may mean :—

1. That the patient is not suffering from typhoid fever.

2. That he is suffering from typhoid fever, but the date is too early for the appearance of the reaction. The reaction sometimes occurs on the fifth or sixth day, usually after the tenth day, and in all but a very small number of cases before the end of the second week. If the onset of the disease (as far as it can be fixed) is less than this the examination should be repeated after two or three days.

3. In a very small number of cases the reaction is delayed still further, and if the patient dies may not occur at all. These cases are usually severe ones and do not present any difficulty in diagnosis. Sometimes the reaction is delayed well into the convalescence in mild attacks, but this is exceedingly rare.

### MACROSCOPIC METHOD.

The macroscopic method can be carried out with a young living culture on agar, or with an emulsion of dead bacilli. The technique is not so difficult as in the previous process and no apparatus is required other than a piece of narrow glass tubing from which to make pipettes.

*Requisites.*—1. A young culture on agar and some

normal saline solution ; or a dead emulsion of typhoid bacilli.

2. Special glass pipettes. These are to be made from a piece of glass tubing which should not be more than  $\frac{1}{8}$  in. in internal diameter and about ten inches long. The central two inches of this tube are to be heated in a flat gas flame until thoroughly softened, then withdrawn, and then the two ends are to be drawn steadily apart. The softened portion is to be drawn out into a long narrow portion (rather thicker than a vaccine tube) about six inches long and of nearly uniform diameter. The full diameter of the tube should pass *rapidly* into this narrow portion ; it should not taper gradually (fig. 12, c). The two pipettes thus obtained are to be broken apart, leaving about three inches of the narrow tubing attached to each. The wide end of the tube is to be plugged fairly firmly with cotton-wool. This serves a double purpose ; it prevents any of the fluid from getting into the mouth, and it offers a certain amount of resistance to the escape of the fluid, and thus renders the pipettes more easy to fill.

*Process.*—Prepare an emulsion just as before, making a drachm or so, and filtering it. An emulsion of dead bacilli obtained from a laboratory should be ready for immediate use. Insert the tip of the pipette into the blood serum (of which there should be several drops), and aspirate it gently into the tube, avoiding air-bubbles ; if these gain access blow out the serum and begin again. It is easier to see what you are doing if you use a piece of india-rubber tubing (such as is used with a hæmocytometer) through which to apply suction.

Having drawn up enough serum to form a column about two inches in length in the narrow portion of the

tube, lay the pipette on its side, and make a mark with ink or with a grease pencil to show how high the serum reaches. Now suck the column of fluid a little way into the tube, and insert the tip of the pipette into the emulsion; suck the latter up the tube until it reaches to the mark. This will give you the same amount of emulsion as of blood serum; the two fluids will be separated by a short column of air. Now withdraw the tip for a moment and suck up another small quantity of air; dip it into the emulsion and suck it up to the mark again. This will give you twice the amount of emulsion as of blood serum, the three portions of fluid being separated by air (fig. 18). Repeat this process until you have nine times the amount of emulsion as of serum; then suck the fluid still further from the tip of the pipette, and seal the latter in a flame. Probably by this time the fluids will have mixed together; if not, tap the tube gently (holding it upright) until the air-bubbles which you have sucked up make their escape and the fluid forms a continuous column.

Fill another tube to a similar height with emulsion (for a control) and place the two side by side in an upright position for twelve hours.

Now examine the fluid in the pipettes. If the emulsion has been made from a living culture the control pipette (*i.e.*, that to which no blood has been added) will probably remain turbid; if an emulsion of dead bacilli has been used it will have become clear, and the bacilli will form a uniform even layer at the bottom of the pipette.

Compare the control tube with the pipette to which blood has been added. If the reaction is negative the appearances will be exactly the same in each; but if the reaction is positive the bacilli will fall to the

bottom in flocculent granular masses, which are similar whether living or dead bacilli have been used. In the former case there is no difficulty in determining whether



FIG. 18.—Showing method of filling pipette.

*a.* Is the mark on the tube. *b.* The serum. *c, c, c.* The columns of emulsion separated by the air bubbles *d, d, d.* *e.* Is the cotton-wool plug.

the reaction is present or absent, for the fluid will be clear if it is positive, turbid if it is negative (fig. 19). The dead emulsion presents more difficulties; you have to compare the granular deposit which occurs in a positive reaction with the more uniform deposit which occurs in the control tube. If there is any doubt the best plan is



FIG. 19.—Positive and negative Widal's reaction (macroscopic method). The fluid in the left hand pipette (negative) is still turbid. The right hand pipette (positive) contains clear fluid and has a sediment at the bottom. The lower portions of the pipette have been removed.

to break off the tip of the pipette and blow out some of the deposit; examine it under the microscope after applying a cover-glass. In a positive case the bacilli will be seen to be collected into clumps, in a negative case they will remain discrete.

This process is a modification of that described by

Wright (*Brit. Med. Jour.*, p. 355, 1898), to which the reader is referred for fuller details.

It is advisable to use a dilution of one in ten if the macroscopic method is used.

## GONORRHŒA.

Nothing is more certain than the fact that gonorrhœa can only be diagnosed by bacteriological methods; and every practitioner is very strongly urged to practise himself in these methods and to employ them in *all* cases. Leaving out of account the confidence which the certainty of a correct diagnosis inspires, there is always the possibility that legal questions may arise, and a practitioner who made a diagnosis of gonorrhœa without employing the only means by which that disease can be diagnosed would make a poor show in cross-examination. Lastly, a bacteriological examination will often tell us that the disease is merely lying latent and is still infective when apparently cured.

The gonococcus chiefly affects mucous surfaces; the urethra in the male, the urethra and cervix uteri in the female, and the conjunctiva in both sexes. These are the regions in which the primary lesion usually occurs, and it may extend by continuity to more distant parts.

In the male it may involve the prostate, the vesiculæ seminales, and the bladder. It is doubtful whether gonorrhœal epididymitis is due to this organism or to another.

In the female the inflammation of the urethra may extend to the bladder. The inflammation of the cervix may extend to the mucosa of the uterus and thence to

the Fallopian tubes (causing pyosalpinx), to the mouths of the tubes (causing local adhesive peritonitis, which probably results in sterility), or to the peritoneum, where it may cause general peritonitis.

The gonococcus may escape into the blood from any of these lesions, and the results of this occurrence are arthritis, ulcerative endocarditis and meningitis; the two latter are rare.

The search for the gonococcus may have to be made (1) in urethral pus from either sex, (2) in pus from the cervix uteri, (3) in pus from the conjunctiva, (4) in pus from the meninges, tubes, peritoneum, or other region, whether removed by operative measures or at a post-mortem examination, (5) in the blood, or (6) in the urine. It is to be noticed that the gonococcus rarely, if ever, attacks the vagina, and that in cases of vaginitis the cervical secretion should be examined.

In the vast majority of cases cultural examinations are quite unnecessary. This is fortunate, for the gonococcus does not grow readily on artificial media. The organism has well marked morphological characters, and the deductions drawn from these characters need only be corroborated in cases of generalised infection or of meningitis, in which the results are to be published (as they should be), and must, therefore, be proved beyond doubt. In such cases the services of a bacteriologist should be called in if possible: or the material may be collected in pipettes with the most careful precautions as to asepsis and forwarded *at once* to a laboratory.

## METHOD OF MAKING THE FILMS.

The pus is to be spread out into thin films at the time at which it is taken, and this is true whether the practitioner intends to make the examination for himself, or is about to send the material to a laboratory. Gonorrhœal pus should never be forwarded dried on a piece of cotton-wool or enclosed in vaccine tubes.

The films are to be made thus:—Take two clean slides and place two or three platinum loopfuls of the pus on the centre of one of them; sterilise the needle and lay it down. Now take the other slide and apply its centre to the pus and allow it to fall on to the first slide by its own weight; do not squeeze the slides together. Then slide them apart, keeping each in its own plane until they are entirely separated. This will give you two excellent films. Allow them to dry and fix them in the flame.

The films may also be made on cover-glasses, exactly the same process being adopted, except that it will be necessary to squeeze the two lightly together. The fixation is accomplished by passing the cover-glasses rapidly through the flame.

These are the methods by which films are spread in all cases; the way in which the pus should be obtained varies somewhat with the nature of the case.

In the male it is advisable to cleanse the meatus and to reject the first drop of pus, taking the second with a platinum loop and proceeding as before. Antiseptic precautions are entirely unnecessary, as no attempt is to be made to get cultures. If the patient is suffering from phimosis, and there is a purulent discharge which



may be due to gonorrhœa, chancre, soft sore, or to a non-specific balanitis, a similar method is adopted, but here many films should be taken, as a prolonged search may be required. If the patient suffers from a slight discharge in the early morning the best plan is to give him two clean slides. These are to be smeared across the meatus whilst wet with the discharge and allowed to dry.

In the female it is necessary to obtain the pus directly from the urethra; it may be expressed by the finger in the vagina. The first drop should be rejected.

If the patient is suffering from cervicitis or endometritis the pus should be taken direct from the cervix, a speculum being used.

It is absolutely necessary that you should spread the films *at once*, even if you are having the examination made at a distance. It is next to useless to send pus dried on linen, cotton-wool, a Volkmann's spoon, or even in a thick layer on a slide. The diagnosis *may* be made from material sent in this way, but the difficulties are much greater, and in some cases the results are less certain.

Preparation of films from conjunctival pus presents no difficulties. The same is true of pus from the tubes or other internal regions, whether it is exposed by operative interference or at a post-mortem examination.

Instructions for the examination of the blood are given subsequently. A considerable number of films should be taken, as the cocci are present in but very small numbers.

The urine may be examined in the female if a local examination is not considered advisable, or in the male to obtain evidences as to whether the disease is cured or not. The morning urine should be examined. It

should be mixed with a small quantity of carbolic lotion or other antiseptic and allowed to settle for twenty-four hours; it is much better to use a centrifuge if one is available. In cases where we require evidences as to cure after an attack of gonorrhœa the urine is examined after gentle massage of the prostate.

### STAINING OF FILMS.

One film is to be stained by a simple stain such as methylene blue or carbol-thionin. The other is to be stained by Gram's method, and then in dilute carbol-fuchsin for *half a minute*. Bismarck brown may also be employed but is hardly as good.

*Examination of films.*—First take the specimen in which the simple stain has been used and examine it with the oil immersion lens. You will see that it shows numberless cells with very irregularly lobed nuclei; these are the pus cells or polymorphonuclear leucocytes. There will also be some flat squamous epithelial cells.

The gonococci will be stained even deeper than the cell nuclei, and will be mostly contained within the pus cells. If you see a cell which contains numerous small blue or violet granules bring it into the centre of the field and examine it more thoroughly, to see whether the granules have the characters of the organism which we are about to describe.

The gonococcus is a large diplococcus, each component of the pair being shaped like a kidney, the hilum being turned toward that of its fellow. Single forms (which may be rounded) and tetrads are sometimes seen. It does not stain by Gram's method, and this is one of its most important features. Another important point is

its arrangement; during the height of an attack of gonorrhœa it is almost entirely intracellular, being contained within the polymorphonuclear leucocytes. Further, several pairs occur in each cell, and the great majority of cells are entirely devoid of cocci.

If the organism which you find possesses these characteristics turn to the specimen which has been stained by Gram's method and counter-stained by carbol-fuchsin (Plate I., fig. 6). In this all bacteria which retain Gram's stain will be coloured violet, while organisms which do not retain it will be red. You must therefore search for groups of diplococci contained within the cells and possessing the above characteristics. They will not be so prominent as in the other specimen, for the cells, nuclei, &c., will be coloured red also, and the contrast is not so great. But if the case is one of gonorrhœa you will find them after a careful search.

#### INTERPRETATION OF RESULTS.

You are justified in considering a case to be one of gonorrhœa if in films made from the pus :—

1. Large kidney-shaped diplococci are present.
2. These cocci occur within the pus cells, and at least four pairs (Foulerton) in each affected cell.
3. The vast majority of cells are entirely free from cocci.
4. The organisms in question do not stain by Gram's method. This is an absolutely essential point.

If cocci which answer to the above description, except that they are not enclosed within cells, the case may still be one of gonorrhœa. The gonococcus is

frequently extra-cellular during the early stages of an attack of urethritis, and, though to a less extent, during its involution, whilst cases sometimes occur in which a considerable number of the cocci lie free during the whole course of the disease.

## CHOLERA.

The diagnosis of cholera can only be made on clinical grounds alone during an epidemic, as other diseases present almost identical symptoms and course. The importance of making a correct diagnosis arises less from the interests of the patient than from those of the general public; if the case is one of true Asiatic cholera the sanitary authorities must be notified and the fullest precautions taken to prevent the spread of the disease. In all suspicious cases a quantity of the rice-water stools (in a bottle sterilised by boiling or by dry heat and *securely packed*) should be forwarded at once to a public laboratory. Meanwhile the diagnosis may be established with a fair amount of certainty by the following simple tests:—

1. Take a platinum loopful of the dejecta and spread it in a thin film on a clean slide; dry, fix, and stain with carbol-fuchsin for three minutes; wash, dry, and mount.
2. Prepare another film and stain by Gram's method.

## EXAMINATION OF THE FILMS.

The spirillum of Asiatic cholera is about half as long as a tubercle bacillus, or rather longer, and much

thicker. It is slightly curved, hence the name of the "comma bacillus," the comparison being to a German comma; it looks very like a caraway seed (Plate II., fig. 5).

In the carbol-fuchsin specimen vast numbers of these curved rods will be seen; probably few other organisms, if any, will be present if the case is one of true cholera. Two or more rods may often be found joined together with their concavities turned in opposite directions, giving the whole the appearance of a very elongated spiral. In the stools (but not usually in cultures) the individual rods have frequently a parallel arrangement, presenting the appearance of "shoals of fish swimming up stream."

If you see these appearances examine the Gram specimen. Very few organisms will be visible, as the cholera vibrio does not retain the stain when treated in this way.

If vibrios having the above characters are present proceed as follows:—

3. Take two or three small flasks (preferably sterilised by heat) and add to each 100 c.c. of water, 1 gramme of peptone, and  $\frac{1}{2}$  gramme of common salt; boil thoroughly and allow to cool. This forms a culture medium in which the cholera vibrio will grow very rapidly and other organisms far more slowly.

Inoculate each flask with a loopful or two of dejecta; plug each with cotton-wool and incubate for 8 to 12 hours at 37° C. If cholera vibrios are present the cultures will conform to the following tests:—

a. There will be a film on the surface. This will be more marked after a few hours longer.

b. This scum will present the microscopic appearances described above, except that the vibrios are

usually somewhat straighter than those which occur in the stools and the "fish in stream" arrangement is not marked. They will not stain by Gram's method.

c. The addition of a small quantity of pure strong sulphuric acid will give a pink or crimson tint. This is the "cholera-red" reaction and is caused by the action of sulphuric acid on indol in the presence of a minute quantity of a nitrite; many other organisms (*e.g.*, the bacillus coli) produce this colour *after* the addition of a nitrite, very few without it. The cholera vibrio produces nitrites as well as indol.

#### INTERPRETATION OF RESULTS.

In a case in which the above phenomena are observed, the inference that the patient is suffering from true Asiatic cholera is so strong that the authorities should be notified and the fullest precautions taken.

A case in which they are absent is almost certainly not one of true cholera.

#### PLAGUE.

The bacteriological diagnosis of plague should be made by an expert; not because it is difficult, but because so much hinges upon it, at least in this country. A brief account of the method by which a practitioner who was unable to obtain expert help might proceed may not be out of place.

The plague bacillus is a short and rather thick rod which occurs in vast numbers in the bubo, in the blood,

and in the internal organs. It does not stain by Gram's method, and when stained by other processes it often exhibits a characteristic *polar staining*, the ends of the bacillus being coloured deeply, whilst the intervening portion remains colourless (Plate II., fig. 4). It might be mistaken for a diplococcus; it could not be mistaken for the pneumococcus (to which it has some resemblance) as that organism stains by Gram. Degenerate forms which resemble cocci, &c., often occur in cultures, but are seldom met with in the body during life.

The diagnosis may be made by an examination of fluid aspirated from the bubo or of the blood. In bubonic cases the former method should always be adopted, as the bacilli are present therein in vast numbers and generally in pure culture; the amount of fluid which has to be removed is very small, even if cultures have to be taken.

When this is not the case two films should be made in the way already described, fixed and stained, the one by dilute carbol-fuchsin or Löffler's blue and the other by Gram's method. If the bacilli are present they will appear as short oval rods which may or may not exhibit the polar staining; if the specimen has been stained for the proper length of time (about 2 minutes) most of them will do so, but in any case it will most likely be present in a few. The Gram specimen will not show these rods; there may be a few pus cocci present as a secondary infection.

The blood is examined by any of the methods to be described subsequently, and a very careful search made, as the numbers of the bacilli may be comparatively scanty.

If a careful examination of stained films made from

a bubo does not show the organisms having the above characters, it is probably safe to say that the case is not one of plague.

### SOFT SORE.

A very strong case has been made out in favour of the view that the bacillus described by Unna and by Ducrey is actually the cause of soft sore, though the proof does not rest upon the solid ground demanded by Koch. The bacillus has never been cultivated outside the body, and the chief proof of its pathogenicity rest upon the fact of its invariable presence in true soft sores. It is hardly correct to say that it is invariably absent from other sores, unless, indeed, we extend somewhat our conception of what a soft sore really is; for the author has found bacilli which were indistinguishable from these in sores which were exactly like those which follow herpes preputialis, though more severe than is generally the case. But it is quite possible that future research will show that the bacillus varies greatly in virulence, and that it is only under certain circumstances that it produces typical soft sores. At present all we can say about the diagnostic value of the organism is that its presence in the secretion from an ulcer affords strong evidence that the case is really one of chancroid, and that its absence almost certainly nullifies such a diagnosis. It is scarcely necessary to say that syphilis and soft sore (or gonorrhœa and soft sore) may be inoculated at the same time, and the lesions appropriate to both diseases may be present simultaneously. The bacillus in question is a short straight rod, less than a quarter the length of the tubercle bacillus, and



not much longer than the bacillus of influenza. It is rather thick in proportion to its length, its length being only about three times its breadth. It does not stain by Gram's method; it stains, indeed, with some difficulty, and powerful stains (such as dilute carbol-fuchsin or Löffler's blue) should be used. It is best demonstrated in films made from the deeper parts of a typical soft sore, for the superficial parts contain bacteria of all sorts, and the recognition of Unna's bacillus is not easy unless it is obtained in large numbers.

The bacteriological examination for this bacillus has most often to be made in cases of urethral sore or of a sore concealed beneath a phimosis. The method of obtaining the specimen is the same in both cases. A fairly stiff platinum loop is inserted beneath the prepuce or into the urethra, and moved gently about until the most tender spot is found. This should be scraped as forcibly as the patient will allow, and the loop withdrawn, care being taken that the mass of secretion is not wiped off in so doing. Several films should then be made, the secretion being rubbed up on the slide with a drop of water. They should be stained with either of the stains mentioned above for five minutes or more; it is an advantage to warm them gently. They are then rinsed in water, dried, and mounted, and examined thoroughly with an oil-immersion lens.

When buboes occur in the course of soft sore the pus they contain should be examined for this organism as soon as they are opened; the interest in this is chiefly scientific, for opinions are divided as to whether they are caused by this germ or by pyogenic bacteria. In three cases examined by the author, it was found (in very scanty numbers) on one occasion, staphylococci on another, and no bacteria of any sort in the third.

## RINGWORM.

Examination of the hair and scales from the skin are of very great value to the dermatologist. It settles conclusively the question whether a patient is or is not suffering from ringworm, and often gives important information as to prognosis; and may enable us to say whether the disease has probably been derived from a human source or has been contracted from one of the lower animals.

Where the mere diagnosis of ringworm is in question an examination of the hair or scales after soaking in liquor potassæ is often sufficient. The materials are placed in a drop of the solution on a slide and covered with a cover-glass; after a quarter of an hour or so the specimen is carefully examined under the microscope, using a  $\frac{1}{8}$  in. lens and a small diaphragm. The spores appear as spherical or oval highly refractile bodies which can hardly be mistaken for anything but fat globules. This possible source of fallacy may be removed by soaking the hair in ether before applying the liquor potassæ.

This is a rapid and simple method, but it does not enable us to diagnose the nature of the fungus with certainty, except in very typical cases, and when spores are scanty they are readily overlooked. It is a very great advantage to employ some method of staining. These are by no means difficult, though they are somewhat tedious. Two processes, both modifications of Gram's method, will be given.

## MORRIS'S METHOD OF STAINING HAIRS, SCALES, &amp;c.

*Requisites.*—1. Anilin gentian violet, carbol-gentian violet, or carbol-fuchsin.

2. Gram's iodine solution.

3. Anilin oil to which sufficient iodine has been added to give it a deep mahogany colour.

4. Anilin oil.

5. Xylol. This is not necessary unless the specimens are to be kept permanently.

6. Blotting paper.

7. Slides, cover-glasses, and balsam.

- *Process.*—Take the hairs from the edge of the suspected area, and cut off all the free portion except a piece about a quarter of an inch long. The root and the part of the shaft next to it are all that are to be examined. Several of these hairs can be stained at the same time.

Stain in a watch-glass full of stain, or on a slide for a quarter of an hour or longer; it is an advantage to warm the stain gently, and it will penetrate better if the hairs have been previously washed in ether, though this is not absolutely necessary.

Remove the hairs from the stain and place them on a slide; if already on a slide pour off the stain. Dry them with blotting paper and pour on the iodine solution; allow it to act for five or ten minutes. It is an advantage to blot the specimen after a minute or two and apply a fresh lot of the solution. Blot thoroughly.

Now decolorise in the solution of iodine in anilin oil. This should be poured off and replaced occasionally, and the specimen examined under a low power of the

microscope. When the decolorisation appears to be complete pour off the solution and replace it by anilin oil; allow this to act for a minute or two.

If the specimen is not to be kept permanently it may now be mounted in balsam and examined at once. If it is to be kept the anilin oil must be washed out by several applications of xylol. Mount in balsam.

Adamson's method gives even better results, but is somewhat more tedious.

*Requisites.*—1. Liquor potassæ.

2. Dilute alcohol—about 15 per cent.

3. Anilin gentian violet or its substitutes.

4. Gram's iodine solution.

5. Anilin oil.

6. Xylol.

7. Blotting paper.

8. Slides, cover-glasses, and balsam.

*Process.*—Prepare the fragments of hair as before, rejecting the free portions. Place them on a slide, add a drop or two of liquor potassæ and apply a cover-glass. Allow the liquor to act for a quarter of an hour or twenty minutes.

Now place a large drop of dilute spirit at one edge of the cover-glass and a piece of blotting paper at the opposite edge; this will suck up the potash and the spirit will run in and replace it. After a few minutes lift off the cover-glass and wash the hairs gently in more spirit. This will harden them. Dry. If epithelial scales are being examined they may be fixed to the slide or cover-glass by heat in the usual way.

Stain in anilin gentian violet for half an hour or less.

Pour off the stain, blot gently, and pour on Gram's iodine solution. Allow this to act for five minutes. Blot again.

Decolorise with anilin oil, pouring it off and applying a fresh lot from time to time. The process may take an hour or more, and the specimen should be left under the microscope and examined occasionally.

When the decolorisation is complete (*i.e.*, when the color is seen to be present in the fungus only), blot gently, and wash thoroughly with xylol. Mount in balsam.

The specimens are to be examined under an  $\frac{1}{8}$  in. objective. A higher power is unnecessary.



FIG. 20.—*Microsporon Audouïni*.\*

There are certainly three, and possibly more species of ringworm which occur in England, and the fungus of favus is closely allied and is demonstrated by the same process.

The *microsporon Audouïni* is the most common species of ringworm fungus in this country, being responsible for about eighty or ninety per cent. of all cases. It is the small spored fungus, and it may be distinguished by the fact that its spores are arranged in an irregular

\* Figs. 20, 21, 22 are from Curtis's "Essentials of Bacteriology" (Longmans).

mosaic and not in chains (fig. 20). It does not invade the interior of the hair, but forms a sheath of spores and mycelium outside the submerged portion of the hair; this sheath projects for a short distance above the surface of the scalp and may be seen with the naked eye.

This fungus usually attacks the scalp in children. It is very rare in adults, and it rarely attacks other regions than the head. It is thought by some to be always caught from a human case of the disease, but there are reasons for thinking that it may be derived by infection from the horse or cat.



FIG. 21.—*Trichophyton endothrix*.

The most important clinical fact about the micro-*sporon* Audouini is that ringworm caused by it is extremely intractable and may run a prolonged course in spite of the most skilful treatment.

The *trichophyton endothrix* attacks the interior of the hairs, and forms long chains. Its spores are somewhat larger than those of the preceding species, but the difference is not great; the organism is most easily recognised by the chain-like arrangement of its spores,

and by the fact that they lie within the hair, the cuticle of which usually remains intact (fig. 21).

This fungus is a rare cause (in this country) of ringworm of the scalp, and the disease caused by it appears to be somewhat easier to cure than that due to the microsporon, though opinions on this point are divided. It also attacks other regions of the body, causing tinea circinata or eczema marginatum.

It always appears to be derived from a human case, and never by infection from animals.



FIG. 22.—*Trichophyton ectothrix* from a case of kerion.

The *trichophyton ectothrix* (like the microsporon) forms a sheath round the outside of the hair, to which it is closely applied, like the bark to a tree. The spores are about as large as in the preceding species, and are arranged in chains; this fact, together with the position of the fungus with regard to the hair, will enable a diagnosis to be made (fig. 22).

In some cases this organism does tend to invade the hair, and in this case the name "endo-ectothrix" is applied to it.

This fungus is a rare cause of ringworm of the scalp, and the disease it causes is readily cured. It also causes ringworm of the body and of the beard region, which the endothrix does not attack. According to Sabouraud (to whose work on these organisms we owe most of our knowledge on the subject) kerion is always caused by this organism, but this is not generally accepted. It appears, however, to be a fact that suppurative lesions (folliculitis, kerion, &c.) are *usually* caused by this fungus.

This species is often derived from one of the lower animals, especially from the horse, cat, and dog.

Favus is caused by a closely allied organism, the *achorion Schönleini*. This may be demonstrated by either of the processes already described. It consists of mycelial threads which are not continuous, but are composed of short rods. The whole has an appearance which has been compared to that of the metatarsal bones, and this resemblance is increased by the fact that sometimes several filaments radiate from one point, forming the "favic tarsus."

The identification of the variety of organism which is present does not usually present great difficulties. The first point to be looked to is the arrangement of the spores. If these are present in an irregular mosaic the microsporon is present; if they form filaments somewhat resembling those of a streptococcus the organism is a trichophyton. To settle which it is look to see whether the cuticle is present, and whether the fungus invades the interior of the hair. This can usually be determined by focussing up and down until you see an "optical section" of the hair in question. It is important not to be deluded by the fact that the fungus which lies on the outside of the hair will appear to be inside it if a surface view only is taken.



## PART III.

**COLLECTION AND EXAMINATION OF  
CERTAIN MORBID MATERIALS.**

Methods of collection and examination of:—

Fluids from the pleural cavities.

Fluids from the synovial cavities.

Fluids from the meninges (lumbar puncture).

Pus.

Materials obtained at post-mortem examinations.

The blood, including:—

1. Enumeration of red corpuscles.

2. Enumeration of leucocytes.

3. Preparation of blood films.

4. Examination for the parasite of malaria.

5. Bacteriological examination.

Solid materials by sections.

**THE COLLECTION OF PATHOLOGICAL  
EXUDATES.**

A bacteriological examination of the inflammatory exudates which collect in the various cavities of the body often yields important information as to the nature of the morbid process, suggests treatment, and influences our views as to the prognosis of the condition. This is especially the case with the fluids which collect in the pleura, the membranes of the brain

and cord, and the joints. In some cases all the needful information may be obtained by the examination of stained films, cultures being unnecessary; and in these cases no anti- or aseptic precautions (other than those which are dictated by the interests of the patient) are necessary. But in the greater proportion of cases this is not enough, and cultures must be obtained. To this end it is *absolutely essential* that the most scrupulous precautions should be taken against contamination of the fluid by the organisms which are constantly present in the air and in the skin, or the results will be worthless. The precautions taken must be as complete as those which are used before an operation upon a joint. Indeed, a fresh precaution has to be taken, for whereas the presence in the skin of a small quantity of an antiseptic would not be detrimental to a surgical operation, it might, by getting into the fluid, nullify a bacteriological examination. Hence the skin must be aseptic and free from any antiseptic chemical.

The technique, as far as aseptic precautions are concerned, is as follows:—The skin at the region to be punctured is first thoroughly cleaned with soap, hot water, and (if the patient can stand it) a nailbrush. Then layer after layer of some reliable antiseptic lotion is painted on, each layer being allowed to soak in before the next is applied. The most suitable lotions for the purpose are perchloride of mercury (1 in 1000) biniodide of mercury, (1 in 500 of methylated spirit) or carbolic acid (1 in 20).

After being allowed to act for at least ten minutes the skin is to be thoroughly cleansed with methylated spirit; this should be rubbed in with a piece of cotton-wool and should be poured copiously over the area. The operation may now proceed.

Where possible it is preferable to apply a dressing of lint soaked in one of the above lotions (which need be of only half the strength) for a few hours.

The puncture may be made by means of some sort of exploring syringe, or by means of a hollow needle without any means for aspiration. The former is used for the removal of fluid from the pleura or synovial cavity, the latter in performing lumbar puncture. But it is necessary that the whole of the instrument used should be rendered sterile by heat; chemical antiseptics are quite inadmissible. In the case of a hollow needle no difficulty occurs; some hypodermic syringes, however, will not stand boiling, and these are useless for the purpose, as the apparatus which is to be used must be boiled for at least five minutes.

We shall now deal with the most important cavities of the body, describing the methods to be employed in the investigation of the inflammatory exudates which they may contain, and the inferences which may be drawn from the results of the examination,

## THE PLEURA.

There is but little to be said about the method to be employed in the collection of fluid from the pleural cavities. The most careful antiseptic precautions are to be taken, and the region to be punctured should be decided by consideration of the physical signs.

The examination of the fluid thus obtained may be either microscopical, cultural, or by injections into animals. If the latter are required (and inoculation should be performed in all cases where a tubercular

origin is suspected) a considerable quantity of the fluid—an ounce or more—should be enclosed in a bottle which has been sterilised by boiling, and forwarded at once.

Where the diagnosis is to be made by cultural methods, and the cultures are not to be made on the spot, the fluid is best stored or sent to a laboratory in pipettes. These are to be filled from the syringe direct; the needle is to be removed and the end of the pipette (sterilised by being passed through the flame) is passed into the fluid and filled by gentle suction at the other end. Each end is then sealed in a flame, care being taken not to heat the fluid. Two or three such tubes should be sent.

*Clear* fluid from the chest rarely, if ever, shows any micro-organisms on microscopical examination. Cultures are usually sterile; where streptococci or pneumococci are found the inflammation is likely to pass on into suppuration. The great majority of these cases of "simple" acute pleurisy are really due to the tubercle bacillus, but their true nature can only be demonstrated by inoculation experiments.

Where any question arises as to the nature of a case of simple pleurisy the practitioner is recommended not to attempt the examination himself, but to send samples of the fluid in pipettes to a laboratory; if animal experiments are necessary a larger quantity of fluid should be sent in a sterilised bottle.

*Purulent* pleurisies (empyemata) may be caused by many organisms, the most common being the pneumococcus, streptococci, staphylococci, and the tubercle bacillus.

The pneumococcus is readily demonstrated by a microscopical examination, the method to be employed being the same as that previously described.

The pus in these cases is thick and creamy, and of a greenish colour; after it has stood for some time a thin layer of a greenish fluid appears upon the surface.

When an empyema is due to the pneumococcus *alone*, no other organisms being present, the prognosis is distinctly better than in cases in which other organisms are present, and the patient has been known to recover after simple aspiration.

The streptococcus is also readily demonstrated by a simple microscopical examination; it grows readily on agar, forming small round colonies which do not tend to coalesce and are more opaque in the centre than in the periphery.

The pus is not generally very thick, and has a yellow colour. It separates into two layers, the upper transparent layer being much more abundant than is the case with pneumococcic pus.

This is the commonest and most dangerous form of empyema. Thorough drainage is essential.

Staphylococcic empyemata, according to Netter, are very rare; the single case in which he found the staphylococcus alone was secondary to ulcerative endocarditis. He also states that when this organism is found in the pus tubercle bacilli are often present as well.

The prognosis of these cases, therefore, appears to be bad.

The tubercle bacillus is responsible for a comparatively small number of cases; and the results of operative interference are not gratifying. The prognosis is worse than in any other form of the disease.

The diagnosis may be made from a careful microscopical examination, but to this end it must be careful, as the bacilli are present in but scanty numbers.

If *no* organisms are found after a thorough micro-

scopical examination, the inference is that the case is tubercular. If a cultural examination is also negative the inference becomes almost a certainty.

Having these facts in view the practitioner is recommended to proceed to examine cases of purulent pleurisy in the following manner:—The pus is to be withdrawn with a hypodermic needle or exploring syringe, and a few drops deposited *at once* on the surface of a culture tube of agar;\* this is to be incubated at the body temperature.

The microscopical examination is made in the manner described for pus, a simple stain and also Gram's stain being used. The presence of streptococci, staphylococci, and pneumococci will be revealed; bacilli may be present, and in this case it should not be forgotten that the *tubercle bacillus* stains by Gram's method. If no organisms are found in these films, or if there are organisms which resemble the tubercle bacillus in general appearance, another specimen should be submitted to *prolonged* staining in hot carbol-fuchsin and decolorisation in dilute sulphuric acid, and thoroughly searched for the tubercle bacillus. If the result is negative several other films should be searched.

The cultures are to be examined after twenty-four hours' incubation. The pneumococcus will produce tiny colourless colonies on the surface of the agar; the streptococcus forms similar small colourless colonies, but these are distinctly more opaque in the centre;

\* The practitioner is recommended to inoculate the culture directly from the hypodermic needle. The cotton-wool plug is to be withdrawn somewhat and the projecting end thoroughly singed in the flame: the needle (still attached to the syringe) is then pushed through the plug, and a drop or two of the fluid expelled directly on to the surface of the medium. A similar device may be employed where the fluid is collected in a pipette.

staphylococci form an even film like a streak of paint; and the tubercle bacillus does not develop. Films should be made from the cultures, stained and examined. The cultural examination is of great value, but much can be made out by the examination of stained films made directly from the pus.

### FLUIDS FROM JOINTS.

The technique of the process of withdrawing these fluids is exactly the same as in the case of pleurisy; the needle will naturally be inserted at a point where there is definite evidence of the presence of fluid, and where it lies near the surface.

The bacteriological examination is conducted on exactly similar lines. A few drops of the fluid should be allowed to flow on to the surface of a sloped tube of agar, and the culture obtained after twenty-four hours' incubation examined in the manner already described. Films should also be made directly from the fluid and some stained by Gram's method and others by a simple stain such as carbol-thionin.

A great number of organisms may be present; the streptococci, staphylococci, the pneumococcus, gonococcus, and tubercle bacillus are the most important. The coccus which has been described by several observers as the cause of acute rheumatism cannot be considered as of diagnostic importance at present; the same remark applies to the bacillus which is probably the cause of rheumatoid arthritis.

Streptococci are readily distinguished on microscopical examination and may be present even if the fluid is perfectly clear. When they are present in a joint

which is not the seat of a perforating wound they indicate a general infection with the streptococcus, ulcerative endocarditis, &c., and the prognosis is most grave. The author was enabled to diagnose a case of streptococcic septicæmia a few hours after the onset of symptoms by finding numerous chains in a single drop of clear fluid aspirated from the knee-joint. The clinical aspect was at that time very similar to that of severe rheumatism, and the case had been so diagnosed.

In such cases the use of anti-streptococcic serum offers some hope to the patient, and should be tried.

Staphylococci are generally found in cases of arthritis due to perforating wounds, or in the course of a general infection. They may also occur along with the gonococcus in cases of gonorrhœal arthritis.

The pneumococcus occurs in general infection from a primary focus in the lung, middle ear, &c., or the course of ulcerative endocarditis.

The gonococcus occurs in some cases of gonorrhœal arthritis; it may be present in pure culture, or it may be mixed with other organisms, especially the pus cocci. In other cases of gonorrhœal arthritis no bacteria are found, either microscopically or on cultural examination, and in these the bacteria have probably died out before the fluid was withdrawn.

The tubercle bacillus may be found in cases of tubercular synovitis, but it is far more probable that the most careful search will be unsuccessful. If bacilli having the general appearance of this organism are found in the Gram specimen, the carbol-fuchsin method of staining should be applied to a fresh film.

Fluid from a joint may be sterile in cases of tubercular synovitis, gonorrhœal arthritis, synovitis due to an aseptic injury, rheumatism, gout, or rheumatoid arthritis, &c.



## LUMBAR PUNCTURE.

Fluid may be removed from the spinal meninges for a bacteriological or other examination by means of Quincke's lumbar puncture. The information furnished by this means is often of very great value; in fact Osler says that "during the past ten years no single measure of greater value in diagnosis has been introduced." The process is simple, easy, and entirely devoid of danger, and can be carried out without an anæsthetic.

*Requisites.*—1. A suitable needle. In children the spinal meninges will be reached at a depth of 3-4 centimetres (roughly 1-1½ inches) while in adults the depth may be twice as great. The needle should not be less than 2½ inches long for an infant and 4 inches for an adult, and should be sharp and strong. An antitoxin needle will answer every purpose. Aspiration is unnecessary, and no syringe is required.

2. Materials for disinfection of the patient's skin and (if cultures are to be taken) the hands of the operator. Hot water, soap, alcohol, ether, perchloride lotion (1 in 1000).

3. Apparatus for boiling the needle in a dilute solution of washing soda.\*

4. Spray for local anæsthesia. (If used).

5. A test-tube sterilised by dry heat and plugged with dry cotton-wool.

6. If cultures are to be taken the tubes of medium should be inoculated at the time of the operation if

\* If possible the needle should be sterilised by dry heat previous to the operation and kept in a tube plugged at both ends with cotton-wool as in the method recommended for the collection of blood for bacteriological examination.

possible. The medium required will depend to a great extent upon the nature of the organism which is expected. If there are no indications upon this point the most suitable medium is solidified blood-serum, but in default of this ordinary agar will answer well. If the case is thought to be one of cerebro-spinal fever the most suitable medium for the cultivation of the specific organism (Weichselbaum's diplococcus intra-cellularis) is alkaline 5 per cent. glycerin-agar, and a couple of tubes of this medium should be at hand, as well as blood-serum or ordinary agar.

*Process.*—I. *Preliminary.*—As in removal of fluids for bacteriological examination from other parts of the body, it is better if the skin can be sterilised some hours before the operation and a pad soaked in an antiseptic fluid kept on the area until the last moment. This is sometimes impracticable, and the process will be described as if it were performed at a single visit.

Put the needle to boil in a weak solution of washing soda, and proceed to the disinfection of the patient's back. When the needle has boiled for ten minutes remove the vessel from the flame and allow it to cool without removing the needle.

Place the patient on his left side and find the processes of the second, third, and fourth lumbar vertebræ. A line drawn between the upper points of the iliac crests usually cuts the spine at the upper edge of the spinous process of the fourth lumbar vertebra. Scrub the skin in this vicinity with soap and hot water; wash the region with alcohol and then with ether and allow it to dry; paint on several layers of perchloride lotion, allowing each to soak in before the next is applied; cover the region with a piece of lint soaked with lotion and proceed to disinfect your hands.

Lastly, pour some alcohol on to the skin of the patient's back to wash off the excess of the antiseptic.

2. *Operation.—Position.*—Get the patient (still lying on his left side) to draw up his knees so as to flex his back somewhat, and to turn partly over on to his face. It is scarcely necessary to say that the operator must not touch the patient, as his hands have now been sterilised.

Identify the processes of the third and fourth lumbar vertebræ and mark the centre of the space between them by means of the index finger or thumb of the left hand. If local anæsthesia is to be employed freeze the skin round a point about one-third of an inch to the right of the middle line, opposite the spot marked by your left finger or thumb. Take the needle in the right hand, holding it like a pen, and enter it at a point level with the centre of the interspace, and 1 cm. (a little less than one-third of an inch) to the right of the middle line. Direct it *forward, slightly upwards, and slightly inwards*, and press it in with a steady and uniform pressure; this must be applied accurately in the axis of the needle, or the latter may bend and take a wrong direction.

If the needle strikes against bone withdraw it almost completely and push it on again after changing its direction slightly. If bone is again encountered it may be advisable to try again in the interspace between the second and third processes.

3. *Collection of fluid and inoculation of media.*—The first few drops of fluid which escape may be stained with blood; in this case it should be rejected. Allow a few drops of the fluid to flow *directly* on to the surface of the media without touching the glass. Collect also some of the fluid (1-4 drachms) in the sterilised empty tube.

If no fluid flows through the needle it is *presumptive* evidence against the presence of acute meningitis. A "dry tap" may, however, occur from plugging of the needle with fibrin, or from its point coming in contact with a nerve root (Osler), and in some cases of meningitis the purulent exudation is too thick to flow through the needle.

4. *Examination of the fluid.*—*a. Naked eye.*—When meningitis is present the fluid is always more or less turbid, and some observers hold that the turbidity is greater in proportion to the severity of the case. Osler has pointed out that the fluid may be alternately turbid and clear, being clear during the remissions, and turbid during the exacerbations of the disease. Blood-stained fluid may occur in meningitis or from hæmorrhage into the cerebral or spinal meninges apart from inflammation. The presence of clear fluid affords strong evidence of the absence of meningitis, but in tubercular meningitis the amount of turbidity may be very slight.

*b. Microscopical.*—Prepare films of the exudate in the manner recommended on p. 131 if the fluid is thin and watery; if it is thick and purulent treat it like ordinary pus. Stain by any of the methods recommended for the examination of the blood (Jenner's stain being most convenient) and examine.

The presence of leucocytes (except in very small numbers) indicates meningitis. If the bulk of the leucocytes are lymphocytes (indicated by their small size, large, circular, deeply staining nuclei, and absence of granules) the presumption is that the case is one of tubercular meningitis. In meningitis due to other bacteria the chief cell is the polynuclear leucocyte; this may be recognised by its larger size, its twisted (apparently multiple) nucleus, and, if

the staining method has been appropriate, by the presence in its protoplasm of minute granules which stain with eosin. The fluid may also contain red blood corpuscles and shreds of fibrin.

This examination is hardly necessary except in cases in which a tubercular origin is suspected.

*c. Chemical.*—Cerebro-spinal fluid removed from a person who is not suffering from meningitis contains a very minute amount of albumen, while when the meninges are inflamed the quantity is greatly increased. The method of testing these small amounts of albumen are hardly within the reach of practitioners; if a considerable amount of fluid has been obtained a small quantity should be tested by heat and acetic acid and the amount of opacity noted.

*d. Bacteriological.*—The chief organisms which cause acute meningitis are given in the following table, which is modified from one given by Osler :—

**Primary** (*i.e.* not dependent on an obvious lesion elsewhere in the body).

1. Cerebro-spinal fever—

- |             |                                      |
|-------------|--------------------------------------|
| a. Sporadic | } <i>Weichselbaum's diplococcus.</i> |
| b. Epidemic |                                      |

2. Pneumococcic—

- |   |                        |
|---|------------------------|
| a. Pneumococcic infection of meninges alone<br>not dependent on disease of distant parts of<br>the body.              | } <i>Pneumococcus.</i> |
| b. Pneumococcic infection of meninges occurring<br>as part of a general septicæmia without<br>obvious primary lesion. |                        |

**Secondary.**

A. To direct extension from local disease of the cranium, middle ear, fossæ, spinal column, &c.

*Pneumococcus.*

*Staphylococci.*

*Streptococci, &c.*

B. To septicæmic infection due to disease in a distant part of the body.

a. Pneumococcic—

Secondary to pneumonia, endocarditis, &c.

*Pneumococcus.*

b. Pyogenic—

Secondary to abscesses, &c., and occurring as a part of a general infection.

*Staphylococci.*

*Streptococci, &c.*

c. Gonorrhœal—

Secondary to gonorrhœa.

*Gonococcus.*

d. Tubercular—

Secondary to tuberculosis of other regions.

*Tubercle bacillus.*

e. Miscellaneous—

Secondary to typhoid fever.

*Typhoid bacillus.*

Secondary to influenza.

*Influenza bacillus.*

Secondary to anthrax, &c.

*Anthrax bacillus, &c.*

Of these the organisms which are most likely to occur are Weichselbaum's diplococcus, the pneumococcus, the tubercle bacillus, streptococci, and staphylococci. The examination for these bacteria may be carried out by means of stained films or cultures.

*Preparation of films.*—If the fluid is thick and purulent, films should be prepared, dried, and fixed in the ordinary way. If the fluid is thin and watery it should be allowed to stand for some hours. A certain amount of coagulation will take place and the sediment which collects will contain the bulk of the micro-organisms. This sediment should be removed by means of a pipette or platinum loop and films prepared from it. The subsequent examination will depend to some extent

upon the nature of the organism which is probably present; for general purposes stain one or more films with Löffler's methylene blue (two minutes), wash, dry, mount, and examine.

Streptococci and staphylococci will be readily recognised by their morphological characters. If diplococci are present they may be pneumococci, Weichselbaum's diplococci, or gonococci. Stain a film by Gram's method and counterstain in dilute carbol-fuchsin in the method described for the gonococcus. Pneumococci will retain the violet stain, while Weichselbaum's organism and gonococci will be coloured red.

Weichselbaum's *diplococcus meningitidis intracellularis* is now generally considered to be the specific cause of cerebro-spinal fever. It is a medium-sized diplococcus, the components of the pairs being approximately hemispherical in shape and having their flat surfaces turned towards one another. This organism has a strong resemblance to the gonococcus, and this likeness is increased by the facts that it is decolorised by Gram's method and that it is mostly contained within the polynuclear leucocytes of the inflammatory exudation. The two differ in their cultural characters and in their pathogenicity to animals. If any question should arise as to which of the two is present in the meningeal exudation in a case in which no cultures have been taken, some help may be afforded by the fact that the diplococcus meningitidis often occurs in the *nasal secretion* in cerebro-spinal fever. It is scarcely necessary to say that other evidence of gonorrhœal infection should be sought for.

Still's diplococcus of posterior basic meningitis cannot be distinguished from the diplococcus meningitidis by its morphological characters alone, and many bac-

teriologists think the two organisms are in reality identical.

*The rarer causes of meningitis.*—The bacilli of typhoid fever, anthrax, influenza, &c., may also be recognised in the methylene blue specimen, and should be identified (if possible) by a careful study of their morphological appearances and reaction to Gram's stain.

If no organisms are found in the methylene blue specimens after a careful search, and if the characters of the fluid are such as indicate that meningitis is present, the presumption is that the case is one of tubercular meningitis. Films should be stained in the method already described (p. 72) and carefully searched; the bacilli are present in very scanty numbers, and many films may have to be examined before one is found.\*

*Cultural examination.*—The tubes which have been inoculated by allowing the fluid to drop directly on to the surface of the medium are to be incubated for twenty-four hours at the body temperature. Streptococci, staphylococci, pneumococci, and the rarer organisms will probably have developed by this time, and will have formed colonies such as have been previously described. Weichselbaum's diplococcus forms (on blood serum) "round, whitish, shining, viscid looking colonies with smooth, sharply defined outlines which attain a diameter of one to one and a half millimetres in twenty-four hours." The colonies on agar are similar but slightly larger, and the growth may become confluent.

\* Lenharz adds a shred of clean cotton-wool to the fluid. This sinks slowly to the bottom, and is withdrawn after some hours, spread on a slide, dried and stained for tubercle bacilli. The author has had no experience of this method, but Mr. Leedham-Green informs him that it is of considerable value.



If no colonies appear on blood-serum or agar at the end of forty-eight hours the case is probably due to the tubercle bacillus or the gonococcus. In some cases of cerebro-spinal fever the diplococci in the exudate are all dead, and cultures remain sterile.

#### INTERPRETATION OF RESULTS.

The discovery of Weichselbaum's diplococcus indicates that the case is one of cerebro-spinal fever. The chief importance in making the diagnosis (apart from the fact that it may throw light upon the occurrence of several cases of meningitis within a short space of time by proving the existence of an epidemic) arises from the fact that the prognosis is decidedly better than in other forms of meningitis.

Meningitis due to the pneumococcus may arise from dissemination from pneumonia or other pneumonic lesion, by spreading from the middle ear, &c., or may be primary. The examination of the exudate throws no light upon this point, and the cause of the infection must be sought for on ordinary clinical lines.

Tubercular meningitis is proved by the presence of tubercle bacilli in the fluid, and is indicated by sterile cultures, absence of bacteria from the stained films, and predominance of lymphocytes.

The other varieties of meningitis do not call for special mention.

The chief value of lumbar puncture to the *surgeon* is that it enables him to diagnose a concomitant meningitis (which would negative an operation) in cases of lateral sinus thrombosis and cerebral abscess. The fluid usually becomes bloody within twenty-four hours of a fracture of the base of the skull or laceration of the

brain. This may assist in the diagnosis of obscure injuries, or of the cause of a case of unconsciousness in which no history can be obtained.

Hæmorrhage into the meninges is indicated by the withdrawal of blood-stained fluid, but it must be remembered that the first few drops may contain a small quantity of blood which has entered the needle during its passage through the tissues, while the rest is clear. Blood-stained fluid may occur in meningitis, and should be submitted to a full examination for leucocytes and bacteria.

*Transmission of fluid to public laboratory.*—If the practitioner is unable to examine the fluid the best method to adopt is to collect it in a long and narrow test tube (sterilised by dry heat) and to seal the neck of the tube. Or he may forward it in a bottle sterilised by boiling and plugged with a cork which has also been boiled, but this method is not so good.

## THE COLLECTION OF PUS.

When a simple microscopical examination has to be made the collection of pus presents no difficulties, as the few bacteria which may gain access from the skin or the air will not lead to error. The case is otherwise where cultural examinations have to be made, or where the material has to be transmitted to a laboratory. Here the material should be collected in a pipette. This is to be at hand when the abscess is opened; both ends are to be broken off and passed through the flame two or three times. The pipette should then be held by an assistant or put with the end which is to be inserted into the pus projecting over the side of the table so as not to come into contact with any object.

When the abscess has been opened a considerable quantity of pus should be allowed to flow out, and the sterilised pipette is then to be passed through the incision (care being taken to avoid contact with its sides) and the pus carefully sucked up into the bulb. The fluid thus obtained may be used to inoculate cultures there and then, or both ends of the pipette may be sealed in the flame and the pipette sent to a laboratory.

### THE EXAMINATION OF PUS.

The organisms which may cause pus are extremely numerous, the most important being streptococci, staphylococci, the pneumococcus and the gonococcus, the bacilli of typhoid fever, tuberculosis, and glanders, the bacillus coli communis, the bacillus pyocyaneus (the organism which produces blue pus), and the fungus of actinomycosis. In the majority of cases the organism which is present in a given sample of pus can be determined by a microscopic examination of films prepared in the usual way and stained by a simple stain, such as carbol-thionin. A specimen should also be stained by Gram's method and the results compared.

When cultural examinations are required they had better be carried out in a public laboratory. If the practitioner should desire to carry them out for himself he had better make stroke cultivations on agar in the manner described on page 22, and incubate them for twenty-four hours at the temperature of the body. The appearances of the colonies will be similar to those described as occurring in cultures made from the blood, to which the reader is referred. It is to be noted, however, that the gonococcus will not grow under

such circumstances unless the surface of the medium has previously been coated with blood.

Another method is to make gelatin plates. This is a very simple matter if the materials are at hand.

*Requisites.*—1. Two or three tubes of gelatin.

2. Two or three sterilised Petri's dishes.

3. A platinum needle—a loop will be best.

*Process.*—Inoculate a gelatin tube in the manner described on p. 22, and then melt it by immersion in warm (not hot) water.

Distribute the pus throughout the melted gelatin by rolling the tube between the hands, and by tilting it from side to side. Do not shake, and do not let the melted gelatin touch the cotton-wool plug.

Take a loopful of the gelatin and transfer it to a second culture tube. Melt the gelatin in this and mix as before. Proceed to inoculate a third tube from the second one if you think it probable that the pus is very rich in organisms.

Now take the first tube and singe the projecting part of the wool plug and heat the mouth of the tube in order to destroy any germs which may be upon it; allow it to cool.

Place the Petri dish on the table in front of you and raise the lid sufficiently to allow you to insert the end of the test-tube; do this, and tilt the latter so that the melted gelatin flows into the dish. Immediately replace the lid, and tilt and roll the dish until the gelatin forms an even film over its whole lower surface. Place it on a flat table to set. Repeat the process with the other tubes. Incubate at about 20° C. for two or three days. Examine the dishes, placing them on the stage of the microscope and using the low power. Each organism will have grown into a small colony, which will resemble

those which are described in the section on the blood. There will be slight differences, but not enough to lead to error if the examination of the colonies is supplemented by an inspection of stained films.

The pneumococcus, gonococcus, the fungus of actinomyces, and the tubercle bacillus will not grow on these plates; and the bacillus of glanders will grow feebly, if at all.

In a day or two longer the plates will, in some cases, be found to have undergone a decided change. If liquefying organisms are present the colonies will soon become depressed below the general surface of the medium and will be surrounded by haloes which consist of liquefied gelatin. This will happen with the staphylococci and the bacillus pyocyaneus; not with the streptococci, the typhoid bacillus, nor with the bacillus coli.

The bacillus of blue pus can readily be distinguished from the staphylococci by its morphological appearance (it is a slender rod) and by the fact that the gelatin round the colony is coloured blue or bluish-green, the growth itself being nearly white.

#### INTERPRETATION OF RESULTS.

The information which is obtained by a study of the bacteria in pus is of more scientific interest than of practical importance. It is the situation of the collection of pus rather than the bacteria causing it which influences treatment and prognosis. A list of the more important results which are produced by the chief pyogenic bacteria may be of interest.

*Staphylococci* are the chief producers of localised suppuration in the skin—such, for instance, as that

which occurs in boils, carbuncles, impetigo, folliculitis, &c. They may cause abscesses in *any* part of the body, and may also give rise to general infections, ulcerative endocarditis, &c.

*Streptococci* usually cause spreading inflammation of the type of erysipelas or cellulitis. They are common causes of osteo-myelitis and suppurative and septicæmic or pyæmic processes connected with the puerperium.

In general infections which are due to streptococci the use of anti-streptococcic serum may offer the only hope for the patient.

The *pneumococcus* often produces suppuration in connection with the respiratory system, especially empyema. It also causes many cases of suppurative otitis media and meningitis.

The *bacillus of typhoid fever* sometimes causes abscesses in connection with the bones after (sometimes long after) typhoid fever. It has been found in other suppurative conditions, *e.g.*, empyema.

The *tubercle bacillus* gives rise to "cold abscesses," usually in connection with bone. The suppuration which occurs in the walls of phthisical vomicæ are due to other bacteria, chiefly streptococci and staphylococci.

The *bacillus of glanders* only causes suppuration in the specific lesions of the disease when these run an acute course.

The *bacillus coli communis* is the chief cause of suppuration occurring in connection with the abdominal viscera, especially of peritonitis due to perforation of the intestine and appendicitis. It frequently attacks the urinary passages, causing cystitis, &c.

The *bacillus pyocyaneus* causes blue pus, usually in connection with the skin or subcutaneous tissues.

The *fungus of actinomycosis* has been dealt with already.

## COLLECTION OF MATERIAL AT POST-MORTEM EXAMINATIONS.

The saprophytic bacteria which occur in such vast numbers in the skin and alimentary canal during life undergo very rapid multiplication after death ; hence, in cases where bacteriological examinations have to be made the *sectio* should be performed as soon as possible after death.

The materials which should be examined in all cases are the heart-blood, the spleen, and the liver, and the following methods are to be employed.

The *heart-blood* should be collected in the method which has been described previously (see p. 42), and cultures may be made upon the spot, or the pipettes sent to a laboratory.

The *spleen* may usually be examined in the same way. If it is so firm and hard that no fluid rises into the pipette, it should be treated in the same way as the liver.

Cultivations should be made from the *liver* at the time when the autopsy is performed. The organ should be cut in half, and a small portion of the cut surface deeply seared with a hot iron. This area is then to be perforated with a stout platinum needle and the culture media inoculated at once.

If the material has to be sent to a distance and no culture tubes are at hand a different course must be adopted. The simplest way is to cut out a cube of liver substance from the centre of the organ, and to sear *every part* of its surface with the flat of a red hot knife. The block (which may be about as large as a lump of sugar) must be dropped at once into a sterilised bottle and sent to the laboratory where the examination is to be made. Another plan is to sear the surface of the

block and then to tie a piece of string round it and dip it quickly into melted paraffin (a candle will do) and allow the coating to set ; the dipping is to be repeated several times, and the specimen (string and all) may then be packed without further precautions. In any case it must reach the laboratory as soon as possible.

Where cultural examinations are not required small portions of the organs should be placed in a suitable hardening fluid as soon as possible.

Other solid organs are treated in the same way. Fluids (pus, the contents of cysts, pericardial or other fluid, &c.) should be collected in pipettes in the manner adopted for the heart-blood.

## EXAMINATION OF THE BLOOD.

A clinically complete examination of the blood includes :—

1. A determination of the number of red corpuscles present per cubic millimetre. (Normal numbers being 5,000,000 in the adult male and about 4,500,000 in the female).
2. A determination of the number of leucocytes present per cubic millimetre (the normal is between 4,000 and 10,000).
3. A determination of the amount of hæmoglobin expressed as a percentage of the normal amount.
4. An examination of stained specimens to ascertain the presence or absence of abnormal corpuscles, and the relative proportions of the leucocytes present.

In addition to these it is sometimes necessary to make :—

5. A determination of the presence or absence of parasites.



## ESTIMATION OF THE RED CORPUSCLES.

The best apparatus for the estimation of the number of corpuscles (whether red or white) is the Thoma-Zeiss hæmocytometer. It should be provided with two pipettes, and costs about thirty-six shillings.

Examine the pipettes. Each has a small bulb containing a little glass ball and a stem which is graduated into several parts below the bulb, and has a single transverse graduation above it.

The pipette intended for use in counting the leucocytes may be distinguished by the fact that it has the figure 11 over the single transverse graduation above the bulb.

There are two sorts of pipettes used for counting the red corpuscles. In the one form the stem below the bulb is divided into ten parts, the upper one (nearest the bulb) being marked 1, and the middle one 0.5 (fig. 23, S). In the other one the same portion of the stem is graduated into three portions numbered  $\frac{1}{100}$ ,  $\frac{1}{50}$ , and  $\frac{1}{20}$ ; the figure mentioned first is placed nearest the bulb. These pipettes are used in the same way, and it is quite immaterial which is obtained; we shall describe the use of the first form.

The *rationale* of the method is this:—Blood is sucked up to one of the divisions on the lower part of the stem, and then an inert diluting fluid is drawn up to the single mark above the bulb, and the two mixed by rotating the whole apparatus for a minute or two. This gives us a dilution of blood of definite strength, the exact amount of dilution depending upon the amount of blood which was taken. Thus, if blood had

been drawn up to the figure 1 we should have a dilution of 1 in 100, while if blood had been drawn up to the figure 5 the dilution would be .5 in 100, or 1 in 200, and

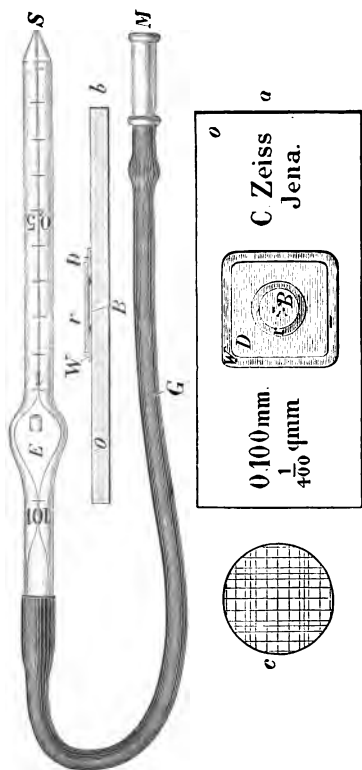


FIG. 23.—Thoma's Haemocytometer.

so on. In the case of the other form of pipette the dilution is read off directly from the figures on the lower stem.

The diluted blood thus obtained is spread out in a film of a definite known thickness on the slide supplied

on the instrument (fig. 23, *a*). This is ruled in squares, and the squares are of known size. The amount of blood lying upon each square is thus known, and the number of corpuscles which lie upon it being counted under the microscope, all the data for the calculation are obtained.

In blood examinations it is absolutely necessary that all points in the technique should receive the most careful attention, or the result will be worse than useless. For this reason we shall describe each step in the process at some length, and advise the practitioner to make several estimations before placing any reliance whatever on his results.

*Requisites.*—1. The hæmocytometer.

2. A needle suitable for obtaining a small quantity of blood. A Hagedorn's needle is the very best that can be used, and an ordinary triangular surgical needle will answer very well.

3. Diluting fluid. There are a good many formulæ for this, and some are rather complicated. Isotonic saline solution (common salt .5 per cent.) will answer perfectly; it is advisable to add to it a small quantity of some stain, methyl violet being the best, although gentian violet will do very well. This colours the *leucocytes*, so that they are readily distinguished from the red corpuscles.\*

4. A microscope having an  $\frac{1}{8}$  inch lens which will focus through the thick cover-glass supplied with the hæmocytometer. If the examination is not to be made by the bedside a strong india-rubber band a little shorter than the pipette should be carried.

\* The following formula is better:—Distilled water, 160 c.c.; glycerine, 30 c.c.; sodium sulphate, 8 grams; sodium chloride, 1 gram; methyl violet, a trace.

## PROCESS.

1. *Pricking the patient.*—The blood may be procured from the convex border of the lobe of the ear or from the lateral surface of the last phalanx of the finger. The advantage of the former situation is that the pain is very slight, the skin being thin, and that the patient cannot see what you are doing and is not likely to start at the critical moment. It is to be recommended for children and nervous women. The advantage of the finger is that the skin is free from hairs, and these are objectionable in the preparation of film preparations by the cover-glass method; an additional advantage is that the patient can put his hand into the position most convenient to you and you have not to lean over him.

The area of the skin to be punctured is washed with soap and water and then with pure water, and wiped dry. No other sterilisation is necessary unless you are also taking blood for bacteriological purposes. The needle is sterilised by being passed slowly through the flame of a spirit lamp or Bunsen's burner; the area of skin to be pricked is taken between the finger and thumb of the left hand, and a rapid and fairly deep stab made with the needle. The skin is then released and a drop of blood allowed to exude; this is wiped away and the next drop which oozes out is used for examination.

The skin must never be pinched when blood is being withdrawn; the blood must always be allowed to flow out naturally.

2. *Filling the pipette.*—The degree of dilution is determined by the number of corpuscles per cubic millimetre which you expect to find. If the patient is anæmic use

1 in 100; if he has approximately the normal number of corpuscles, or if you have reason to think that they be present in increased quantities, use a dilution of 1 in 150 or 1 in 200.

Having decided upon the degree of dilution insert the tip of the pipette into the drop of blood lying on the skin, take the bone mouth-piece attached to the india-rubber tube in your mouth, and suck the blood up to the appropriate mark. If air bubbles gain access blow the blood out and begin again. If you over-shoot the mark remove some of the blood by touching the tip of the pipette against some absorbent cotton-wool. Now remove the pipette from the blood and wipe off the excess with your finger; prevent blood from flowing out by placing the tip of your tongue in the aperture of the bone mouth-piece. Place the tip of the pipette in the diluting fluid; a small quantity should be poured out into a watch-glass or other suitable vessel, so as to avoid any possibility of allowing some blood to escape into the stock bottle and invalidating a subsequent observation. Suck the diluting fluid slowly into the pipette until it reaches the single mark above the bulb; it is best to rotate the pipette between the finger and thumb as you do so.

Now remove the pipette from the diluting fluid, preventing escape of fluid from the bulb by placing the tip of the tongue on the aperture of the mouth-piece as you do so; then place the tip of the finger over the aperture of the pipette (fig. 23, S) and proceed to mix the contents by rotating the pipette and by turning it over and over. It is hardly necessary to say that it is useless to shake it.

If the examination is to be made at a distance remove the india-rubber tube and stretch an india-rubber band so as to close both apertures of the pipette.

3. *Preparation of the specimen.*—The slide which is supplied with the instrument consists of a thick and perfectly flat slip of glass (fig. 23, *o*) on which is cemented a glass square having a round hole in its centre (*W*). In the centre of the hole thus left there is a circular disc of glass (*B*); this inner disc is made of glass which is exactly  $\frac{1}{10}$  of a millimetre thinner than that of which the outer glass is constructed. When the whole cell is covered with a perfectly flat cover-glass (*D*) there will, therefore, be a space exactly  $\frac{1}{10}$  of a millimetre between the lower surface of this cover-glass and the upper surface of the central disc; this space is to be filled with the diluted blood.

Slide and cover-glass are to be wiped clean with a soft handkerchief moistened with water and then thoroughly dried; there must not be the minutest particle of dust on any part of the surface.

The slide and cover-glass being ready, mix the contents of the pipette as you did before (this must always be done immediately before making the specimen, no matter how carefully it had been done a short time previously) and blow out about half of the fluid in the bulb; this is to wash the diluting fluid out of the lower part of the stem. Now clip the india-rubber tube firmly between your finger and thumb so as to prevent the access of air, and, therefore, the escape of fluid, and wipe the tip of the pipette from all fluid; this may be done with the forefinger. Place the tip of the pipette on the centre of the central disc of the slide and relax your pressure on the india-rubber tube so as to allow a *small* drop of fluid to escape; this is perhaps the most difficult part of the process, and the exact amount which must be allowed to fall on to the slide can only be learnt by experience.

Cover the slide in this way :—Place your finger at the side on the glass square on the slide, and apply the cover-glass, letting it rest against your finger ; lower it gently in place with a needle or other suitable object. When it is in place press it gently with the needle at each corner in succession, and look at it obliquely so as to see the light reflected from the surface. If the slide and cover-glass are in sufficiently close contact you will see Newton's rings (looking like the eye of a peacock's feather) round the point at which you are applying pressure. If you do not see this the inference is that there is some dust between the slide and cover-glass ; you must clean both and begin again.

If you have taken the right amount of fluid the drop should extend exactly to the edge of the central glass disc, but should not run over into the "moat" (*r*). If this happens, or if there are any bubbles under the cover-glass, you must begin again. If the drop does not quite extend to the edge of the central disc no great harm is done.

4. *Focussing the specimen*.—This is somewhat difficult for beginners, and merits a short description. Place the slide under the microscope, taking care to get it accurately centred, and examine it with the low power. You will find that the central disc is ruled into squares like a chess-board (*c*). Get these squares into the centre of the field.

Do not forget you are dealing with an unstained object ; use a flat mirror and a small diaphragm. The examination is often easier if artificial light is used.

Now turn on the high power ( $\frac{1}{8}$  in. or  $\frac{1}{7}$  in.) and screw it downwards until it *almost* touches the cover-glass ; look down the microscope and focus gently upwards, using the fine adjustment and keeping a careful lookout for the rulings.

Some  $\frac{1}{8}$  in. lenses focus too near the object to be of any use. If this is the case you must either get an objective specially for the purpose, or a cover-glass which is hollowed out in the centre. These can be bought from the same place as the hæmocytometer.

If the rulings of the slide are indistinct they may be darkened by rubbing them with a very soft lead pencil.

5. *Counting the corpuscles.*—Move the slide about until you have come to one corner (preferably the left upper corner) of the ruled area. You will see that each fifth space is marked off by a line running down its centre; this is to guide the eye and facilitate counting. If you exclude the spaces which are thus marked with a double line the whole area will be marked out into a series of large squares, each consisting of  $4 \times 4 = 16$  smaller squares (fig. 24). It is convenient to count the smaller squares in these groups of 16. *At least* a hundred of the smaller squares, *i.e.*, six of the large groups and four small squares, should be counted.

In counting one of the smaller squares it is convenient to begin with the corpuscles which are lying in the middle of the square, and then to count those which are lying on the lines. In dealing with these you count those which are lying on the *upper* and *left hand* lines as being within the square, and those that are on the *lower* and *right hand* lines as being without it; if you like you may reverse this, but you must keep to the same method throughout (see fig. 24).

A few white corpuscles will be met with in every case, while if the blood was taken from a patient with leucocytosis or leucocythæmia there will be many. They may be distinguished from the red corpuscles by their greater refractivity, or, if a stain has been used in the diluting fluid, by their being faintly tinged. It is



scarcely necessary to say that they should not be counted.

6. *The calculation.*—The best way of calculating the number of corpuscles present from the data thus obtained is the following:—

First add up the number of corpuscles in all the squares which you have counted and divide the sum by the number of squares counted. This gives the average in each square.

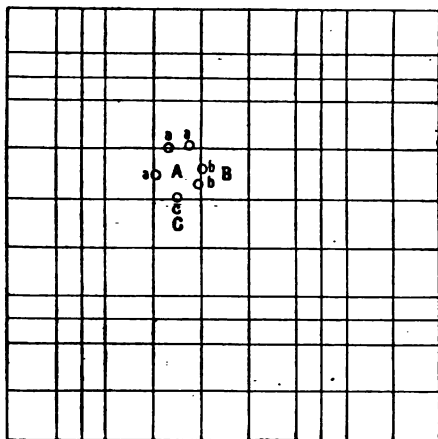


FIG. 24.—Showing method of counting red corpuscles: *a, a, a*, are counted in square A; *b, b*, in B; *c*, in C.

Now the space enclosed between each square and the cover-glass above it is  $\frac{1}{10}$  of a millimetre deep,  $\frac{1}{20}$  of a millimetre wide, and  $\frac{1}{20}$  of a millimetre long; its cubic capacity is therefore  $\frac{1}{10} \times \frac{1}{20} \times \frac{1}{20} = \frac{1}{4000}$  of a cubic millimetre. Therefore the  $\frac{1}{4000}$  part of a cubic millimetre contains the number of corpuscles which we have already found as the average.

But the square contained *diluted* blood; if the amount

of dilution was 1 in 100 the amount of blood contained in the space over each square was  $\frac{1}{100}$  part of  $\frac{1}{4000}$  of a cubic millimetre.

Therefore, the number of corpuscles which has been determined as being the average per square is contained in  $\frac{1}{4000}$  of  $\frac{1}{100}$  of a cubic millimetre of undiluted blood, the dilution being taken as 1 in 100.

Hence the number of corpuscles in one cubic millimetre of undiluted blood is obtained by multiplying the average per square by the number which expresses the dilution (in this case 100) and then by 4000.

It may be expressed as a formula, thus:—

If  $n$  is the total number of corpuscles counted,

$s$  is the number of squares counted,

and if the dilution is 1 in  $d$ .

Then the number of corpuscles per cubic millimetre is  $\frac{n}{s} \times d \times 4000$ .

*Example.*—Suppose that we have counted 100 squares and have found that they contain 1200 corpuscles. Then the average per square is 12.

Then  $\frac{1}{4000}$  of a cubic millimetre of diluted blood contains 12 corpuscles.

Or,  $\frac{1}{100}$  of  $\frac{1}{4000}$  of undiluted blood contains 12 corpuscles, supposing the dilution was 1 in 100.

Therefore, one cubic millimetre of undiluted blood contains  $12 \times 100 \times 4000 = 4,800,000$  corpuscles.

Or by the formula:—

Number of corpuscles per cubic millimetre:—

$$\frac{1200}{100} \times 4000 \times 100 = 4,800,000.$$

The beginner is strongly advised to work out the problem at full length until he has become absolutely familiar with the reasons for all the steps.

## ESTIMATION OF THE NUMBER OF LEUCOCYTES.

All the steps are similar to those just described at full length, except that a different diluting fluid is used.

The diluting fluid is one which destroys ("lakes") the red corpuscles, but does not injure the leucocytes. It consists of a .3 or .5 solution of acetic acid (glacial) in water; it is better to add a small quantity of methyl violet or gentian violet, so that the leucocytes are stained and thereby rendered more prominent. This solution is best prepared fresh or at any rate kept in a well stoppered bottle.

The pipette is distinguished from that used for the red corpuscles by its having the number 11 above the bulb. This indicates that if blood be sucked up to the mark 1 below the bulb, and diluting fluid up to the transverse mark above the bulb, the dilution will be 1 in 10, and so on.

The blood should be sucked up to the mark 1 if a great excess of leucocytes is not expected. If the case is one of leucocytosis a greater dilution is better, whilst if there is a great excess of leucocytes (such as occurs in severe leucocythæmia) it is best to use the red corpuscles pipette with a dilution of 1 in 100, but employing the acetic acid diluting fluid.

In counting the squares a different method has to be employed, as a far greater number of squares must be counted. Either you may count *all* the squares on the slide and then make a fresh preparation and count all the squares on it; or you may adopt the following method, which is much quicker and, therefore, since it

permits of more squares being counted in a reasonable time, more accurate.

Having focussed the rulings on the slide move the draw-tube of the microscope up and down until the upper and lower limits of the field of the microscope coincide *exactly* with two of the horizontal lines, and count the number of spaces (each enclosed between two horizontal lines) in the diameter of the field. Using a  $\frac{1}{8}$  in. objective it will be found possible to arrange matters so that these are eight in number, and this will be found convenient, though any other number will do.

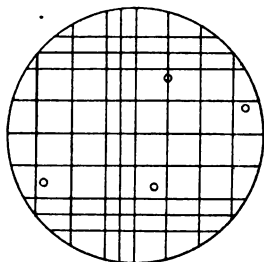


FIG. 25.—Showing field of microscope adjusted so that its diameter is equal to that of eight squares.

The essential thing is that the upper and lower borders of the field shall coincide exactly with the rulings. We will suppose that the number is eight. Then the diameter of the field of the microscope is equal to eight times the length of a side of a square, and its radius is equal to four times the length of a side of a square. The total area of the field of the microscope is therefore  $4 \times 4 \times \frac{22}{7}$ , ( $r^2 \times \pi$ , where  $\pi$  is taken as  $\frac{22}{7}$ ) or 50 and a fraction. Practically, therefore, when we look down the microscope after it has been adjusted in this

way we are looking at 50 squares; and this fact enables us to dispense entirely with the rulings and count over the whole area of the disc with great rapidity. The slide is placed in position and all the cells which are seen in the field counted and the result noted down, or, preferably, dictated to someone else. The slide is then moved on until a perfectly fresh portion of the field comes into view; it is advisable to go too far rather than not far enough. For this purpose (as for a great deal of blood work) a mechanical stage is a great advantage. In this way 2000 squares may be counted in a very short time; it is an advantage, however, to count 1000 squares, *i.e.*, 20 fields, and then to clean the slide and to prepare a fresh preparation from the fluid which remains in the pipette and count another 20 fields.

The calculation is just the same as for the red corpuscles, remembering that the dilution is very different. The average per square will of course be less than unity. The same formula is applicable.

Immediately after use the pipettes must be thoroughly cleaned. The fluid which remains in the bulb must be blown out; and for this purpose, as well as for the subsequent washings, it is an advantage to reverse the position of the india-rubber tube, so that the fluid may be blown out through the upper part of the pipette, this being the wider. The whole pipette must now be filled with water (preferably distilled) and the water blown out. This process is repeated, using absolute alcohol and allowing it to run out of the pipette without blowing it. Lastly, fill the whole pipette with ether, remove the india-rubber tube, replace it with the tube of an ordinary spray (such as is used for scent fountains, throat sprays, &c.) and pump air through until the

apparatus is absolutely dry. You can tell when this has happened by the fact that the ball inside the bulb will emit a clear ringing sound when the pipette is shaken. It is useless to attempt to dry the tube by blowing through it from the mouth.

If blood has coagulated within the apparatus it must be digested out. Fill the whole with an artificial digestion fluid (pepsin and very dilute hydrochloric acid) and place it in a test-tube of the same fluid in a warm place for twenty-four hours. Then try to clean it as before and repeat the digestion if this is impossible.

## ESTIMATION OF THE AMOUNT OF HÆMOGLOBIN.

There is no absolutely satisfactory apparatus for the estimation of the amount of hæmoglobin in the blood at present on the market. Those which are chiefly used in this country are Gowers', and Oliver's. Gowers' is the simplest and by far the cheapest form; the necessary manipulations are very easily learnt, but it is not easy to take exact readings. Indeed, the margin of error is very considerable. Oliver's hæmoglobinometer is a little more difficult to use, but it is somewhat easier to read; its price prevents it coming into universal use. It is to be recommended where *absolute* results are required; for clinical purposes when we wish to see whether a patient is or is not improving under treatment Gowers' will answer quite well.

It consists of two tubes mounted in a small stand. One of these tubes is filled with a jelly tinted to represent the colour of normal blood of a certain

degree of dilution. The other is graduated into a hundred parts, the graduation being such that when 20 cubic millimetres of normal blood are diluted with water up to the 100 mark the colour of the two tubes should be exactly the same. A pipette measuring 20 c.m. and a dropping bottle (which is to be filled with water) are also provided.

*Method of use.*—Place a few drops of water (preferably, but not necessarily, distilled) in the graduated tube.



FIG. 26.—Gowers' Hæmoglobinometer.

Draw the blood in the usual way. Apply the tip of the measuring pipette to the drop and suck gently until the blood reaches up to the mark. Now put the tip of the pipette into the small quantity of water in the bottom of the graduated tube and blow out the blood; suck water up the pipette until it reaches above the mark and blow it out; repeat this process until the blood is thoroughly washed out from the tube.

Place the two tubes side by side on a sheet of white paper in front of a well-lighted window which is not

exposed to direct sunlight; look at them by the light which is reflected from this paper, and add water from the pipette belonging to the dropping bottle, drop by drop, until the colour in the two tubes is exactly the same. Read off the height of the column of diluted blood; this gives the percentage amount of hæmoglobin.

Oliver's hæmoglobinometer differs from that of Gowers' in that the degree of dilution is constant and the colour of the diluted blood is read off by comparison with a series of carefully graduated standards. It consists of (1) a capillary glass tube with thick walls and ground ends, one of which is flat and the other pointed; this tube is mounted in a metal handle, the other end of which serves as a stirrer (fig. 27, *c*); (2) a small cell with an opaque white bottom, and provided with a cover-glass which has a slight bluish tint (*e*); (3) a series of twelve coloured glass discs mounted over an opaque white background (*a*); (4) certain small pink glass discs used as riders; (5) a short glass pipette with an india-rubber nipple at one end and a short length of india-rubber tubing at the other (*d*); the latter fits over the pointed end of the capillary tube mentioned first; and (6) a small wax candle such as is used for Christmas trees. A camera tube lined with a green material is used to screen the eyes whilst the comparison is being made.

*Method of use*.—Prick the patient in the usual way. Apply the polished end of the capillary tube to the drop of blood; this will completely fill the tube, being drawn up by capillary attraction. When quite full wipe both ends of the tube with the fingers and apply the end of the glass pipette (previously filled with water) to the pointed end of the capillary tube. Now



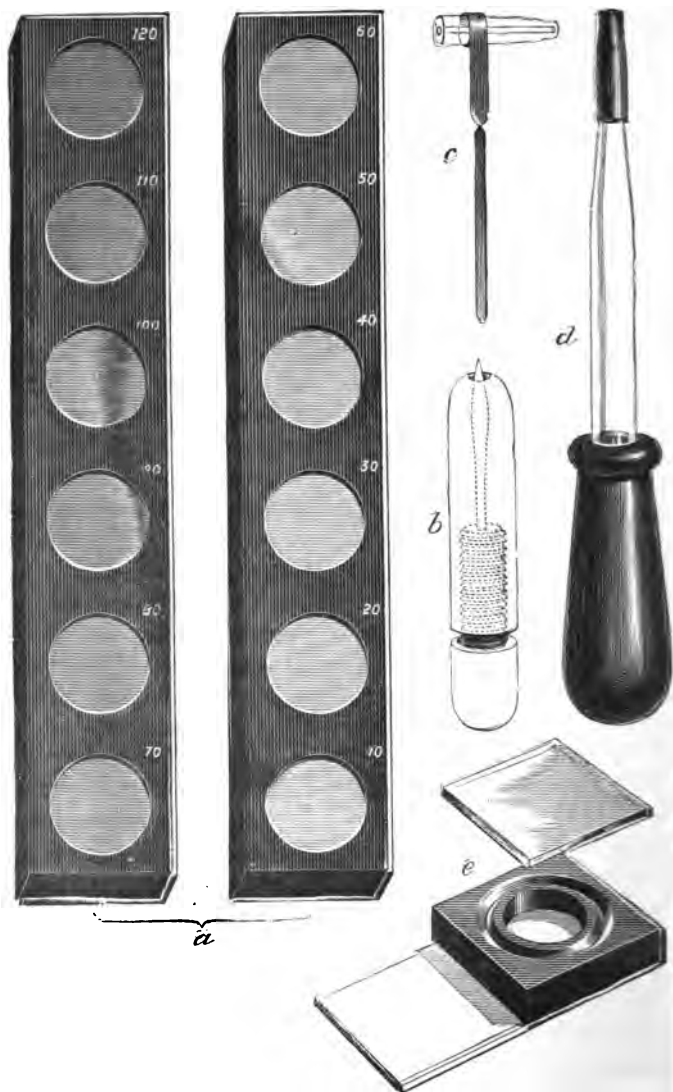


FIG. 27.—Oliver's Hæmoglobinometer.

squeeze the nipple gently so as to force the blood and (subsequently) the water drop by drop into the cell. Interrupt the process occasionally and stir the contents of the cell with the metal handle of the measuring tube. Continue to add water until the cell is *exactly* full; this is the first step which presents the slightest difficulty. Apply the coverglass; this must not enclose any air under it, nor cause any of the diluted blood to flow into the moat round the cell.

The specimen is now ready for comparison with the standards. It is to be taken into a dark room and examined by the light of one of the candles. This is to be placed in front of the observer at a short distance from the specimen and standards, which must lie side by side.

The viewing is best done by means of a camera tube which folds into the box containing the whole apparatus. It terminates in a diaphragm which is perforated by two small holes, one of which is to be placed over the centre of the specimen and the other over the centre of the standard. The latter is to be moved about until a disc is found which nearly or quite corresponds in colour with the diluted blood in the cell. If the correspondence is exact the process is at an end; the number against the disc in question represents the percentage amount of hæmoglobin. If there is no disc which exactly matches the specimen the latter is placed against the disc which is nearest to it but not so deep in colour. For example, if we found that the specimen was darker than the disc numbered 50, but paler than that numbered 60, then it would be placed opposite to 50. A slip of colourless glass is then applied over the specimen, and riders over the standard disc, until an exact match is obtained. If, in the case mentioned

above we had to add a rider marked 5 to the standard to bring about an exact match, the percentage amount of hæmoglobin in the blood would be 55.

It is an advantage to place cell and standards side by side rather than one above the other, for the upper and lower portions of the retina differ in sensitiveness to colour, whilst the sides do not.

## PREPARATION OF FILMS FOR STAINING.

The processes to be described are the same whether we are making films for the purpose of investigating the nature of the corpuscles and cells contained, or for the detection of parasites. If, in the latter case, we are searching for the malaria parasite very good films are necessary, but if we are only looking for bacteria we may use films that are quite useless for a study of the blood itself.

We shall describe two methods, one with cover-glasses and one with slides and cigarette papers.

### I. METHOD WITH COVER-GLASSES.

*Requisites.*—1. Perfectly clean cover-glasses. These must have been cleaned with nitric acid, washed, and then soaked in ammonia, then washed in absolute alcohol and kept in a mixture of equal parts of absolute alcohol and ether. Immediately before they are required for use they must be removed with a clean pair of forceps and dried with an old and soft handkerchief. They may then be passed through the flame.

2. Two pair of dissecting forceps.
3. A needle for taking blood.
4. A platinum loop having a diameter of about one-tenth of an inch is sometimes useful, especially to a beginner.

*Method.*—The patient having been pricked and the first drop of blood wiped away, take up a cover-glass in one pair of forceps, holding the glass by its corner, and touch the drop of blood with it. In doing so take great care not to touch the patient's skin. There should now be a small drop of blood in the very centre of the cover-glass. This is the difficult step; you must not get too much nor too little blood, or the films will be useless. Now take up the second cover-glass in the other pair of forceps and place it on the top of the drop in such a position that the corners of the two cover-glasses do not correspond. Release the upper cover-glass; it will approach the lower one, being drawn thereto by the capillary attraction caused by the presence of a small quantity of fluid between the two.

At this stage you will see whether you have taken the right amount of blood or no. If you have, the drop will spread out, still retaining its circular shape, until it approaches the octagon formed by the intersecting edges of the two cover-glasses; if you have taken too little it will not reach so far; and if you have taken too much it will extend further and the upper cover-glass will float loosely on the lower.

It is necessary to lay great emphasis on the fact that the cover-glasses must *not* be squeezed together, but must simply come together by capillary attraction.

When the drop has ceased to spread take hold of the upper cover-glass with the forceps at a corner opposite to that of the lower cover-glass which you are still

holding with the other pair of forceps; draw the two apart with a steady even pull; they should separate easily, and, if all the stages of the process have been properly carried out, leave you with two perfectly spread films.

Here again you find whether you have taken the right amount of blood. If you have taken too little the cover-glasses will be very difficult to separate; may indeed be impossible to do so without breaking them. If you have taken too much they will separate with great readiness and the blood will spread in uneven smears instead of forming a uniform film.

A word is necessary concerning the method in which the cover-glasses are to be pulled apart. They must always be kept in the same plane; if this is not done, and if the upper cover-glass is lifted from the lower one, the film will resemble the marks left on a knife which has been pressed upon butter and then lifted off; such films are useless.

The blood may be taken by means of the platinum loop, and this is a good plan, as all danger of smearing the cover-glass upon the skin is avoided. If several films are to be taken a number of platinum loops should be provided, as the blood upon them soon coagulates. The exact size of the loop can only be learnt by experiment, and when one has been found to deliver a drop of the right size it should be kept entirely for this work and carefully protected from injury.

## II. METHOD WITH CIGARETTE PAPERS.

*Requisites.*—1. Perfectly clean slides.

2. Some fairly stiff cigarette papers cut in half

longitudinally. Paper which is decidedly ridged or ribbed will not answer.

### 3. Needle.

*Method.*—The patient is pricked and the first drop of blood wiped away as before. One of the half strips of cigarette paper is now held in the right hand, the index finger being placed above the strip and the edges held between the thumb and index finger and the index and middle fingers respectively; this converts it into a gutter, the convex edge of which is downward. The edge of this gutter which points away from you (and which is formed by a machine-cut edge of the paper)

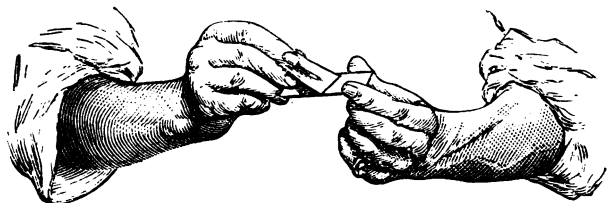


FIG. 28.—Method of spreading films with cigarette paper.

is now dipped into the drop of blood and a small quantity picked up on its lower surface. This lower surface is then placed on a clean slide parallel to one of its shorter edges and about half an inch from it, and pressed gently upon it so as to flatten out the paper gutter; as this flattens out the edge of the drop of blood on its under surface will follow it. The strip of paper is now drawn towards the other end of the slide with a steady uniform movement, and in doing so the drop of blood is spread out into a long uniform film. In this way a film  $\frac{1}{2}$  in. wide and 2 in. long can be made on a single slide. A fresh piece of paper is to be used for each specimen.

The author is of opinion that it is best to adopt the cover-glass method, as he has found that it presents fewer difficulties for beginners; this is not the universal experience, and it is a good plan to try both and adopt that with which you get the best results.

## FIXATION OF BLOOD FILMS.

If films are required simply for bacteriological purposes (*i.e.*, to search them for bacteria) they may be fixed by passing them three times through the flame, just as if they were ordinary films. This, however, is not to be recommended in the study of the cells of the blood or of the parasite of malaria, though it answers very well in skilful hands. Three methods should be learnt, the method by heat, the alcohol and ether method, and the formalin method. Of these the first is necessary if Ehrlich's triacid stain is to be used, but the others are perhaps better for other stains. In addition to these we must mention that if Jenner's stain is used a preliminary fixation is unnecessary, as the fluid fixes the film and stains it at the same time. This is the method of blood examination which is most suitable for practitioners; and it is doubtful whether it is not also the best for the most accurate and delicate scientific research.

### I. METHOD OF FIXATION BY HEAT.

Slides or cover-glasses to be fixed by this method must be exposed to a temperature of 150° C. for

about five minutes—slides requiring a slightly longer time than cover-glasses.

The ideal way is to use a dry air steriliser (see p. 8) to place the films in it whilst cold, to heat up to  $150^{\circ}\text{C.}$ , and then to turn out the gas. In the absence of this apparatus a metal slab or plate such as has been recommended for use in staining the tubercle bacillus answers well. It is mounted upon a tripod stand and the heat applied at one end. After a time the temperature of various portions of the plate are tested by the application of a few drops of water; the point at which the drop assumes the "spheroidal state" (*i.e.*, takes the form of a sphere and does not wet the plate) is about the right point to use. The slides or films are placed at this point for the appropriate time.

## II. FIXATION BY ALCOHOL AND ETHER.

This is very simple; the films are placed in a mixture of equal parts of alcohol and ether for at least half an hour.

This method of fixation is perhaps the best that can be adopted for general purposes.

## III. FIXATION BY FORMALIN.

There are several methods by which the fixative action of formalin can be used for blood work. Of these the use of a mixture of one part of formalin with nine parts of absolute alcohol answers perfectly. The films are immersed in this for half a minute and then washed thoroughly under the tap.

This method of fixation is both good and rapid.



The author is of opinion that it is best to adopt the cover-glass method, as he has found that it presents fewer difficulties for beginners; this is not the universal experience, and it is a good plan to try both and adopt that with which you get the best results.

### FIXATION OF BLOOD FILMS.

If films are required simply for bacteriological purposes (*i.e.*, to search them for bacteria) they are fixed by passing them three times through the flame, just as if they were ordinary films. This, however, is not to be recommended in the study of the blood or of the parasite of malaria, though it may be very well in skilful hands. Three methods have been learnt, the method by heat, the alcohol method, and the formalin method. Of these the first is necessary if Ehrlich's triacid stain is to be used, the others are perhaps better for other stains. In addition to these we must mention that if Jenner's method is used a preliminary fixation is unnecessary, and the film is stained at the same time. The alcohol method of blood examination is the best for practitioners; and it is also the best for the scientific researcher.

Slides  
must be

The film is fixed by heat in the manner of the Giemsa stain and the stain is poured on to it and allowed to stand for five minutes. The film is then removed by blotting paper and then by gentle rubbing in balsam.

The eosinophilic granules are stained green, red blood corpuscles orange, and the neutrophilic granulations bright red. The small lymphocytes which are present in the polymorphous (the neutrophile granulations of the blood) are stained violet. The basophile granulations

are not suitable for the parasite of malaria,

The stain consists of a solution of a compound of methylene blue in methyl alcohol. It must be freshly prepared. Nothing could be more simple in which it is used; no preliminary treatment of the film, the film being allowed to dry and then stained. After a period of from a minute to two minutes the stain is washed off with water and the specimen dried and mounted.

This stain nuclei are stained blue, red blood corpuscles red, and basophile granules red. A striking feature of this stain, and which distinguishes it from other staining methods in which methylene blue and eosin are used is the fact that the fine eosinophile granules of non-nuclear leucocytes take the eosin. I have not been familiar with Jenner's method of staining these cells for the eosinophile granules. No mistake could not arise after one of these cells had been seen for comparison, as its granules are much larger and more prominent.

## STAINING BLOOD FILMS FOR THE INVESTIGATION OF THEIR CELLS.

There are a great many methods of staining blood films, and all depend upon the division of stains into two varieties, the *acid* and the *basic*. All the stains which are used in this branch of histology are salts; and in some of these salts the acid radicle does the staining, in others the basic.

*Acid stains* are those in which the colouring property resides in the acid of the salt. A familiar example is picrate of potash, a yellow stain in which the picric acid is the active ingredient. The acid stains in chief use are eosin, acid fuchsin, and orange G. Substances which stain with an acid stain after suitable exposure to a mixture of an acid and a basic stain are called oxyphile, or, from the frequent use made of eosin as an acid stain, eosinophile.

Basic stains are those in which the colouring property resides in the basic radicle of the salt; they include all the stains which are in use for staining bacteria and they all colour the nuclei of cells. The most important are methylene blue, methyl green, and toluidin.

We shall describe three methods of staining, and these are sufficient for all purposes of diagnosis. They are:—1. Ehrlich's method with his triacid stain; 2. Jenner's method; and, 3. Eosin and methylene used separately.

1. Ehrlich's stain consists of a mixture of acid fuchsin, orange G, and methyl green dissolved in water, glycerine, and alcohol. It is difficult to prepare and should be purchased from a reliable maker. Its use is

very simple. The film is fixed by heat in the manner already described and the stain is poured on to it and allowed to act for five minutes. The film is then washed, dried with blotting paper and then by gentle heat, and mounted in balsam.

Nuclei are stained green, red blood corpuscles orange, and eosinophile granulations bright red. The small eosinophile granulations which are present in the polymorphonuclear cells (the neutrophile granulations of Ehrlich) are stained violet. The basophile granulations are unstained.

This stain is not suitable for the parasite of malaria, nor for bacteria.

2. Jenner's stain consists of a solution of a compound of eosin and methylene blue in methyl alcohol. It must be bought ready prepared. Nothing could be more simple than the way in which it is used; no preliminary fixation is necessary, the film being allowed to dry and flooded with the stain. After a period of from a minute and a half to three minutes the stain is washed off in *distilled* or *rain* water and the specimen dried and mounted.

After the use of this stain nuclei are stained blue, red corpuscles red, eosinophile granules red, and basophile granules violet. A striking feature of this stain, and one which distinguishes it from other staining methods in which eosin and methylene blue are used is the intense way in which the fine eosinophile granules in the polymorphonuclear leucocytes take the eosin. Observers who have not been familiar with Jenner's stain have mistaken these cells for the eosinophile leucocytes; this mistake could not arise after one of the latter cells had been seen for comparison, as its granules are so much larger and more prominent.

Jenner's stain is suitable for a study of the parasite of malaria, which it stains blue. It may be used for the detection of bacteria.

3. Eosin and methylene blue used separately.

In this method the films are to be stained with the eosin first and then with the methylene blue. Its successful application requires a certain amount of practice.

The eosin used must be in watery solution, and the exact strength does not matter; four per cent. is a convenient strength to use. Most specimens of red ink (slightly diluted) will do quite well. The films are to be stained in this solution for three or four minutes; no harm will result if they are left in much longer. They are then washed and immersed in a saturated watery solution of methylene blue. This is the difficult part of the process, for no general rule can be given as to the length of time for which this stain must be applied; it may be ten seconds, or it may be two or three minutes. The only safe way is to stain the film for a quarter of a minute, wash it, and then examine it under the low power of the microscope. If the film is properly stained the nuclei of the leucocytes will be seen as blue points which can be distinguish with great ease with the  $\frac{2}{3}$  inch objective. If they are not visible the methylene blue must be applied for about a quarter of a minute more and the examination repeated. When the nuclei are seen to be well stained the film is dried and mounted.

This process gives results which resembles those afforded by Jenner's stain except that the fine eosinophile granulations in the polymorphonuclear cells are always less obvious and often quite invisible. It is also suitable for malarial parasites and bacteria.

The practitioner is recommended to practice this method of staining, as it does not require any reagents which are not to be found in every well stocked surgery. The watery solution of methylene blue which is used as a counterstain for the tubercle bacillus and some red ink are all that are necessary.

## THE BACTERIOLOGICAL EXAMINATION OF THE BLOOD.

The bacteriological examination of the blood is not of very great diagnostic importance, as it is only in a very few diseases that pathogenic bacteria are present in the circulation in such quantities as renders the search for them in the minute amounts which are withdrawn for examination at all promising.

The chief organisms which have been found in the blood are:—

1 & 2. *Streptococci* and *staphylococci*.—These are found in cases of septicæmia, pyæmia, ulcerative endocarditis, &c.; they always indicate an extremely bad prognosis. The chief importance which attaches to the discovery of these organisms is that it absolutely settles the diagnosis (always provided that there are no errors in technique) and that it indicates whether the use of anti-streptococcic serum is advisable or not; it is useless in cases of septicæmia, &c., which are not due to streptococci.

A word of warning is necessary in the interpretation of results which indicate that staphylococci are present in the blood. These organisms are constantly present in the skin and may be found in film preparations or in

cultures, unless the rigid antiseptic precautions are taken. Streptococci are common contaminations of cultures, but rarely occur in film specimens.

3. *Anthrax bacilli*.—These may be detected with ease and certainty, but they are never found in the blood until it is too late to save the patient.

4. *Tubercle bacilli*.—These are only present in very scanty numbers and are very difficult to detect. The diagnosis of miliary tuberculosis is to be made by other methods, chiefly by that of exclusion.

5. The *pneumococcus* is found in severe cases of pneumonia (probably it might be found in most cases if a sufficiently large quantity of blood were examined) and in septicæmia and ulcerative endocarditis when due to this organism. When found in the blood by ordinary methods it always indicates a bad prognosis and suggests the use of anti-pneumococcic serum. The identification of the organism is easy and certain if they are present in quantities sufficient for them to be found in films.

6. *Typhoid bacilli* are present in the blood in all cases of typhoid fever, but their isolation is difficult and the diagnosis of the disease is made by other methods.

7. The *bacillus of glanders* may be found in acute cases of that disease, but its isolation and identification are matters for an expert.

8. The *influenza bacillus* is present in some, or, according to some authorities, all cases of influenza. It may be searched for in films, but no importance should be attached to a negative result.

9. The *bacillus of plague*.—This organism is often present in the blood in relatively large numbers, and the disease can usually be diagnosed after a careful search through a number of suitably stained films. But

the investigation of a drop of fluid drawn from the bubo (if one is present) permits of an easier and earlier diagnosis. The blood examination is of most value in the pulmonary and septicæmic forms of plague.

10. The *spirillum of relapsing fever* is easily found, for it possesses well marked characters and is present in great numbers. The diagnosis of relapsing fever cannot be made until it has been demonstrated.

11. The *gonococcus* has been found in the blood in a few cases of ulcerative endocarditis. Its detection by cultural methods is very difficult, and the services of a bacteriological expert should be called in if the characteristic cocci are not found in blood films in a case in which the diagnosis of gonorrhœal ulcerative endocarditis is probable, as further information upon this point is greatly needed. We may point out that ulcerative endocarditis, septicæmia, &c., supervening in the course of an attack of gonorrhœa are not necessarily due to the gonococcus. Any pathogenic bacteria may enter through the lesion of the mucous membrane which the gonococcus has caused.

12. The *bacillus coli* is present in some cases of septicæmia.

## EXAMINATION FOR BACTERIA IN FILMS.

This is the easiest method in which bacteria may be found in the blood, and it does not require such a rigid antiseptic technique as is necessary if cultures are to be taken. The films are prepared and fixed in one or other of the methods which we have described, the only point worthy of notice being that the skin must be very



thoroughly cleaned ; it may be scrubbed with soap and a nail brush, using plenty of hot water. The films need not be very thin and even.

The method of staining will depend upon the organism which is likely to be found, and more especially whether it stains by Gram's method. This is so important in this connection that a repetition of a previous table will not be out of place.

### GRAM'S METHOD.

<i>Stained.</i>	<i>Unstained.</i>
Streptococci.	Typhoid bacilli.
Staphylococci.	Bacillus of glanders.
Bacillus of anthrax.	Bacillus of influenza.
Bacillus of tubercle.	Bacillus of plague.
Pneumococcus.	Bacillus coli.
	Spirillum of relapsing fever.
	Gonococcus.

If the organism which is present appears in the first list the staining process is simply that which we have described previously, and the organism will be stained dark blue or violet and the other structures will be unstained.

If the bacteria which are present in the films do not stain by Gram's method the matter is more difficult, for any stain which colours them will colour the nuclei of the leucocytes also. Jenner's stain may be used, or the film may be stained by eosin and methylene blue separately. The organisms will then be stained blue. Carbol-thionin is even more suitable, as the colour which it imparts to the nuclei of the leucocytes is not deep and the red corpuscles are merely tinged. This is

the stain which we recommend for general use, and in cases in which the nature of the organism (if one be present) is entirely unknown.

If bacteria are detected by any of these methods their nature must be recognised by a consideration of their morphological features and staining reactions.

## MALARIA.

The blood in a suspected case of malaria may be examined fresh or in stained films. Of these methods the former is the better and should be used if possible; an examination of stained specimens should also be made and is convenient, as it can be performed away from the patient and at leisure.

Fresh films are made by touching a drop of blood on the patient's finger with the centre of a perfectly clean cover-glass so as to remove an extremely small quantity of blood. This cover-glass is then allowed to fall on to a clean slide so that the droplet of blood may be spread out by capillary attraction and by the weight of the cover-glass, just as is the case in the method of making blood films already described. But the slide is not separated from the cover-glass; they are examined just as they are, a ring of vaseline being painted round the edge of the cover-glass to prevent evaporation.

The specimen is examined with a  $\frac{1}{8}$  in. objective, and a place found in which the corpuscles are spread in a single layer; this part is then searched thoroughly with a  $\frac{1}{18}$  in. oil immersion lens. The parasites are seen as pale irregularly-shaped bodies, with indistinct margins which occupy the interior of the red corpuscles, and

show *amœboid movements* of greater or less rapidity. When the parasites are older they occupy a larger space in the corpuscles, and there are granules of dark pigment around their periphery. These granules are often the first indications of the presence of parasites in the examination of an unstained specimen. At a still later stage the granules will be found in the centre of the corpuscle (the hæmoglobin of which is now almost entirely removed), and the parasite will show segmentation into a larger or smaller number of spores by lines which have a radial arrangement and give the whole

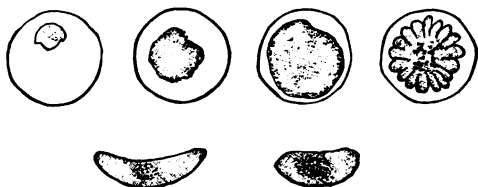


FIG. 29.—Malarial parasites in the blood. The dark area shows the parasite as it appears when stained with thionin.

an appearance resembling that of a marguerite daisy. These are only found when a rigor is imminent.

Crescents are found in the aestivo-autumnal form of malaria; they are crescentic bodies with rounded "horns," and contain a ring of pigment granules in the centre. They cannot be mistaken for anything else, and if a single one is found it affords conclusive proof that the patient has been infected with malaria.

Films for staining are made in the ways already described, and must be thin and perfect. They may be fixed by any of the methods we have recommended, the alcohol-ether and the alcohol-formalin methods being perhaps the best. They may be stained by Jenner's

stain, or by eosin and methylene blue used separately; the parasites are stained pale blue and the corpuscles bright red.

A simpler stain is that recommended by Rees (*Practitioner*, March, 1901) involving the use of carbol-thionin prepared by dissolving 1.5 grammes of thionin in 10 c.c. of absolute alcohol and 100 c.c. of a five per cent. solution of carbolic acid. This is to be kept for at least a fortnight and diluted with four times its bulk of distilled water immediately before use. Staining is complete in about ten minutes. Ordinary carbol-thionin answers very well indeed. Thionin stains the red corpuscles a faint green, nuclei blue, and the parasites an intense purple.

In a suspected case of malaria the search should not be abandoned in less than half an hour, or, in the case of an inexperienced observer, much longer.

A fuller description of the parasite and the differences between the forms which are present in the various forms of the disease is beyond the scope of this work, and the reader is referred to the admirable special number of the *Practitioner* mentioned above.

## COLLECTION OF BLOOD FOR EXAMINATION BY CULTURAL METHODS.

This is a much more difficult matter and should not be attempted unless and until the film method has failed. The difficulty arises from the abundant bacterial flora of the skin; unless the most thorough antiseptic precautions have been taken the results are *absolutely useless*. They are worse than useless, they are

misleading. A case of ulcerative endocarditis, for example, might be due to streptococci, but might be attributed to staphylococci on the strength of an inadequate bacteriological examination. For this reason we are chary of recommending this method of diagnosis in any hands other than those of an expert, and must urge the practitioner not to attempt it unless he is prepared to carry out the most thorough disinfection of the skin.

*Requisites* :—1. A glass pipette such as has been already described. It should be sealed at one end while the other end should be left open and plugged with a loose fitting plug of cotton-wool. The tube must be sterile; if it has been recently made (and we advise the practitioner to make these pipettes for himself) this will be the case; if it has been kept in stock for some time it should be sterilised in the dry air steriliser as an additional measure of precaution. The bulb of the pipette should hold at least 1 c.c., and is to be completely filled with blood.

2. Needle.
3. Soap, nail brush, and hot water.
4. Some reliable antiseptic lotion, such as carbolic acid (1 in 20), perchloride of mercury (1 in 1000), or biniodide of mercury (1 in 500 in methylated spirit).
5. Alcohol. Absolute alcohol is best, but methylated spirit will do.
6. Ether or turpentine; the latter is not so good.
7. Spirit lamp or Bunsen's burner.

*Process*.—Scrub the skin, in the region to be pricked, with the nail brush, using plenty of soap and hot water for ten minutes; then dry the skin and wash it with ether to remove the fat. Next paint on layer after layer of the antiseptic lotion: it is much better to apply a wet

dressings of a more dilute lotion for a few hours. The next step is a very important one, and consists in the thorough washing of the skin with methylated spirit or rectified spirit; this must be continued until every trace of the antiseptic has been removed.

The skin is then to be punctured with a sterile needle; the stab must be a deep one and the blood must flow freely. The first few drops which escape must be rejected.

The end of the pipette is then to be inserted in the drop of blood and placed as near the puncture as possible and the blood sucked very gently into the bulb; great care must be taken lest air should gain access at the same time, for it might contain bacteria which might lead to erroneous conclusions. When the bulb is completely filled *both* ends of the pipette are to be sealed up.

A much better plan is to use a hypodermic needle and to plunge it directly into a vein. The antiseptic precautions are the same as in the former method, and the syringe and needle are to be boiled immediately before use. The vein is made prominent by a bandage applied firmly above the seat of puncture, just as if venesection were to be performed, and the needle thrust obliquely through the skin. If this method is adopted there is much less chance of contamination, and the difficulties of the operation are certainly no less.

Undoubtedly the simplest and best of all methods is that described by James and Tuttle (*Report of the Presbyterian Hospital*, New York, 1898). "A piece of glass tubing  $4\frac{1}{2}$  inches in length and  $\frac{1}{4}$  inch in diameter is drawn out to a tapered end and ground to fit the cap of a rather fine hypodermic needle. The larger end of the tube having been stopped with a cotton plug, the whole

is then placed in a larger tube and both ends of this are similarly plugged with cotton\* (fig. 29).



FIG. 30.—Pipette for collection of blood for bacteriological examination.

\* These may be obtained from F. Ash, Edmund Street, Birmingham.

“The apparatus is then sterilised by dry heat. In using it the inner tube with needle attached is removed; the skin over one of the most prominent veins of the anterior surface of the forearm, near the bend of the elbow is selected, a piece of rubber tubing or a few turns of a bandage being passed round the arm above with moderate pressure, in order to produce distension of vessels. The needle is then plunged into the vessel and generally blood begins to flow by the blood-pressure itself, but any quantity desired may be obtained by making gentle suction either by applying the mouth directly to the end of the tube where it is stopped with cotton, or through the medium of a small piece of rubber tubing slipped over it.

“By the above instrument vein punctures have been made in about 150 cases of a variety of diseases. At no time was any difficulty experienced in obtaining the amount of blood desired, which was generally about 1 c.c. In a few instances it was necessary to try two punctures before securing a free flow through the needle; in no case was there any local reaction whatever at the seat of puncture, nor did the patient complain of pain and annoyance.”

The following points are worthy of attention:—Select for the puncture a vein which is *superficial* (as shown by the blue colour appearing through the skin) not merely prominent, for a deep vein will often slip in front of the needle. Insert the needle very obliquely, with the point directed away from the body. Lastly, remove the bandage as soon as possible after withdrawing the needle, or there may be a considerable amount of hæmorrhage into the subcutaneous tissues. If this should happen place a pad of lint over the puncture and bandage the arm from the wrist upward. No harm will result.



A great advantage of this method is that it does away with the necessity for any elaborate process for the sterilisation of the skin. The authors quoted above found that the results which they obtained were as good after simple cleansing of the skin with soap and water as after the use of antiseptic dressing. Nor is this to be wondered at when we think of the very small chance of any bacteria being carried through the skin and subcutaneous tissues into a vein by a very fine and sharp needle.

The advisability of employing some such method in which the blood is drawn directly from a vein in place of the simple skin puncture is very apparent from the researches of Kühnau ("Zeitschft. f. Hyg. and Infct.," 1890), who made parallel series of experiments by the two methods. He found that in cases in which the blood drawn directly from the vein remained sterile, growth (mostly streptococci or staphylococci) occurred in as many as ninety per cent. of cultures inoculated from skin punctures though the most careful antiseptic precautions were used.

## EXAMINATION OF BLOOD BY CULTURAL METHOD.

This is a matter which is best carried out in a proper laboratory by an expert. If the practitioner attempts to carry it out for himself his best plan is to make a series of inoculations on the surface of agar.\* The tubes

\* The ideal method is to make the inoculation directly into a large quantity of broth and to make sub-cultures on agar later. This method has the advantage that the blood becomes largely diluted, so that any bactericidal substances which it may contain are less likely to injure the bacteria. It is, however, rather more difficult.

are inoculated directly from the syringe, tube, or pipette containing the blood, the pointed end being pushed through the cotton-wool plug (the surface of which must be previously sterilised by having its projecting portion burnt off in the flame) and the blood allowed to flow drop by drop on to the surface of the medium, the tube being turned so that each portion of the medium is covered by a film of blood. If the tube method is used and the blood has been allowed to coagulate the tip of the glass tube carrying the needle should be broken off with a sterile pair of forceps or pliers. The culture tubes are then to be inoculated for twenty-four hours at the body temperature and examined. If they are sterile they are to be returned to the incubator and examined after a further period.

If colonies appear they are to be carefully examined with a lens, and their characters noticed. The organisms which will be most likely to develop are streptococci, staphylococci, anthrax bacilli, pneumococci, typhoid bacilli, the bacillus of plague, or the bacillus coli; the gonococcus may also develop, for it will obtain the hæmoglobin necessary for the development from the blood itself.

*Streptococci* form small white colonies which show no tendency to run together to form a film. The centre of each colony is more opaque than its periphery.

*Staphylococci* form a more or less uniform film, the colonies extending laterally and fusing together. The growth is opaque, and is of a dead white, lemon, or orange colour, according to the nature of the staphylococcus present (albus, citreus, or aureus).

*Anthrax bacilli* form small white colonies having the "barrister's wig" appearance already described.

The colonies of the *pneumococcus* are small flat white points which do not tend to fuse together. They are difficult to see when they are young, and in case of doubt the tube should be returned to the incubator.

The colonies of the *typhoid bacillus* and the *bacillus coli* are whitish and opalescent. They usually have an angular or polygonal appearance when small, and tend to run together when older if they are thickly set. Their discrimination must be left to an expert.

The *bacillus of plague* forms white colonies which are circular or have a crenated outline; they tend to run together and form a uniform film over the surface of the medium.

The *gonococcus*, if it develops, forms very minute transparent colonies which have been compared to droplets of dew. They do not become confluent. This organism will not grow if transplanted on to the surface of ordinary media unless a thick film of blood be previously spread over it.

After cultures have been obtained they are to be examined microscopically by the method described on page 29, and the morphological appearances compared with those of the pathogenic organisms which we have enumerated. It is especially important to test whether the organism which has been isolated, stains by Gram's method or not.

## SECTION CUTTING.

The methods employed in section cutting are somewhat outside the scope of this work, inasmuch as sections are rarely necessary for the purposes of bacterio-

logical diagnosis. The presence of bacteria in the tissues can usually be demonstrated by the simple processes of smearing the cut surfaces of tissues on clean slides or cover-glasses, and treating the films thus obtained by the fixing and staining methods previously described. If, for instance, we have to search for tubercle bacilli in tuberculous glands it is usually sufficient to smear the cut surfaces of the glands on a slide, dry, fix by heat, and stain in the same way as sputum is stained for the tubercle bacillus. If anthrax bacilli were being looked for in the liver or other organ, removed post-mortem, the same method of procedure would be adopted, except that Gram's method of staining would be used. So also for typhoid bacilli in the spleen, where the film would be stained with a simple stain such as thionin or Löffler's methylene blue.

It seems advisable, however, to give a short general account of the processes involved in section cutting, for they are by no means difficult, and do not require very expensive apparatus. Further, the same methods of section cutting are used for investigating the nature of tumours, &c., and this is done already by many practitioners and should be done by still more.

Slices of organs or tissues which are to be cut have first to be *fixed*. The process of fixation consists essentially in the application of some agent which brings about coagulation of the component proteids with as little distortion of the morphological elements as possible; if this step were not carried out the subsequent processes would be liable to cause alterations in the shape, size, and appearance of the cells and fibres. There are two chief methods of fixation, that involving the use of *chemical substances*, and that involving the use of *heat*. The processes which are used in fixing the

tissues *harden* them at the same time; this is necessary, for fresh tissue would yield before the sharpest knife and could not be cut into thin sections. These processes are always carried out, no matter what method of section cutting is to be adopted.

In cutting sections it is necessary that the material should be sufficiently *firm* and *homogeneous* in consistency. The former is secured to some extent by the process of hardening, but a properly hardened block is rarely firm enough to permit of its being cut into sections without further preparation. Further, it almost invariably happens that some parts of the material are firmer or harder than others; and if such a substance were cut the harder parts might be sufficiently firm whilst the softer parts would simply crumble before the knife. There are two methods of overcoming this difficulty—*freezing* and *embedding*.

The *freezing process* is very simple, and it is one which can easily be carried out at home. The sections which it yields are usually quite sufficient for purposes of histological research (the diagnosis of tumours, &c.), but they are rarely sufficiently thin for a proper demonstration of the bacteria which they may contain. The sections are cut more easily by the freezing than by the paraffin process, but they are decidedly more difficult to manipulate.

In the freezing process the block or tissue after fixing and hardening is dipped, or better soaked for some hours, in a thick solution of gum arabic. It is then placed on the plate of a microtome and frozen until the tissue assumes the consistency of fairly hard cheese and can be cut into thin sections.

The *embedding process* should be called the infiltration process; the tissue to be cut is infiltrated throughout

with some firm substance and not merely embedded therein. Two embedding materials are in general use—paraffin and celloidin. The latter will not be described, as it is only necessary for special work and for ordinary purposes cannot compare with paraffin for beauty of results and facility of application.

In the paraffin process the tissue is infiltrated throughout with hard paraffin (such as is used for the better varieties of paraffin candles) so that every cell and every fibre is permeated and supported on every side. To do this requires a number of processes. It would be of no use to immerse the block of tissue in the paraffin just as it is, for the paraffin would not wet it, much less soak into it. The water is first removed; and this is done by soaking the material in absolute alcohol. But alcohol does not dissolve or mix with paraffin; it is therefore necessary to remove it by means of some fluid which will mix with it on the one hand and paraffin on the other. Of these there are many; xylol, chloroform, benzine, cedar oil, and many more are in use for special purposes. Chloroform answers most purposes and is to be generally recommended. The block of tissue is now ready to be soaked in melted paraffin; it is kept in a bath of this substance until the chloroform has been entirely driven off and replaced by the paraffin. The whole is then allowed to cool, is shaped into suitable blocks, and is then ready for cutting.

We shall now describe the processes in fuller detail.

## FIXING MATERIAL FOR CUTTING.

These processes must be understood by all practitioners, even although they do not intend to cut sections

for themselves. It happens to every medical man to find it necessary to send tumours, &c. to a laboratory to obtain a pathological diagnosis; and in very many cases the materials are treated in a way which absolutely prevents good sections from being obtained. Many fixing fluids are in use, and any of them may be selected, but it is absolutely necessary that the material to be investigated should be cut into *small* pieces and put into a large bulk of the fluid *at once*. This is especially necessary in the case of material removed at a post-mortem examination, where the tissues and organs have already undergone alteration.

As regards the size of the slices which are to be placed in the hardening fluid, it is sufficient to say that they should never exceed  $\frac{1}{4}$  inch in thickness, and if perchloride of mercury is used should be even thinner. The other dimensions of the block are of less importance.

The bulk of the fluid in which the block is placed should be at least twenty times that of the block, and it is not advisable to place two blocks in the same vessel.

The fluids which we shall recommend for this purpose are:—

1. Perchloride of mercury in normal saline solution. This is prepared by dissolving common salt in water in the proportion of seven grammes to a litre (about  $3\frac{1}{2}$  grains to the ounce), and saturating this solution whilst hot with perchloride of mercury. The solution must be allowed to cool completely; as it does so crystals of the mercury salt will separate out.

This fluid fixes completely in twenty-four hours, or less, and gives most excellent results. Its powers of penetration are not very great, so that slices of tissue which are to be fixed in it should be thin.

The after-treatment of the blocks fixed in this fluid

must be described briefly. They are allowed to remain in the solution for twenty-four hours and no longer, and are then washed for twenty-four hours in running water to remove the perchloride of mercury. They are then passed through the various strengths of spirit (as will be described subsequently), a little tincture of iodine being added to each to remove any mercury which may still remain. The other steps are the same as those which are used if other methods of fixation have been adopted.

2. Formalin. This should be used in a 5 per cent. solution in water. It yields very good results, and is perhaps the fluid which can be most warmly recommended to a practitioner who is going to send his material to a public laboratory.\* The fluid has very great powers of penetration, and the slices may be much thicker than we have recommended. The one objection to the fluid is that it interferes somewhat with the way in which the sections stain.

3. Alcohol is not a very good fixing fluid, as it tends to cause a good deal of shrinkage. When it is used the blocks should be cut small and placed at once in undiluted methylated spirit.

## SECTION CUTTING BY THE FREEZING METHOD.

Sections which are prepared by the freezing method are rarely as thin as those prepared by one or other of

\* Formalin should not be used for tissues which are to be searched for the tubercle bacillus, as it prevents the decolorising action of the acid.



the infiltration processes, but are prepared very rapidly, and are often sufficient for diagnostic purposes, where rapidity is the first consideration.

The blocks of tissue must be hardened before being cut, any of the above fluids being applicable; where alcohol is used it must be washed out in water, as it will not freeze. Where more rapid work is required the best method is a modification of the old boiling process, as revived by Mr. Strangeways Pigg. The slices of tissue from which sections are to be cut are thrown at once into boiling water and allowed to boil vigorously for two or three minutes; the water must be actually boiling when the tissues are added, and the bulk used should be large as compared with the block. The tissues are then rapidly cooled by being thrown into cold water, and are then ready for cutting. The outer surface of the block should be rejected.

This method of fixation leads to a little distortion of the tissues and alters any blood which they may contain, but it is very good for diagnosing tumours. It is invaluable in the post-mortem room, and for diagnosis of the nature of a tumour during operation. In skilful hands a section may be cut, stained, mounted, and a diagnosis made in ten minutes.

A microtome is necessary for the successful cutting of sections, and the Williams and Swift pattern are those in general use for the freezing process. We shall recommend the practitioner who intends to take up this branch of work to procure a Cathcart microtome, which is exceedingly cheap (it costs about a guinea) and answers admirably. The great advantage of this machine is that it will serve for cutting sections in paraffin as well as for frozen sections.

The blocks of tissue which are to be cut are dipped

in a thick and syrupy solution of gum arabic; if time is no object it is a great advantage to soak them in this for several hours. A block is then placed on the corrugated plate of the microtome and frozen by means of the ether spray which impinges upon it. When the

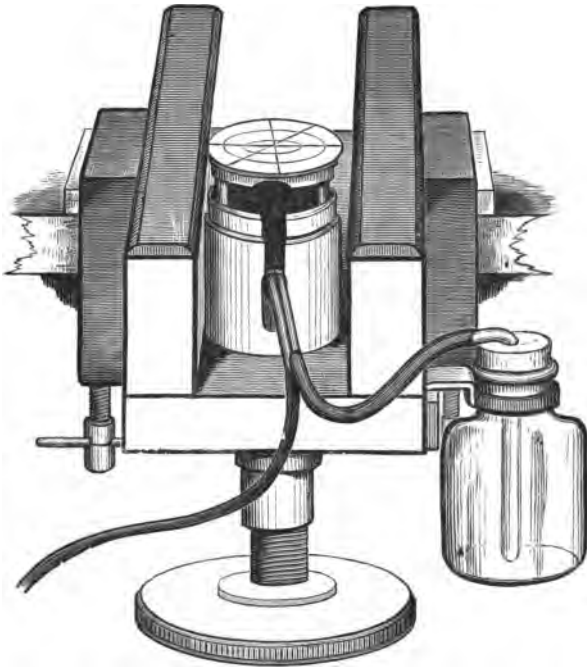


FIG. 31.—Cathcart's microtome arranged for cutting frozen sections.

mass is nearly frozen a section is taken off by means of a razor which is ground flat on one side, or the special knife which may be obtained with the apparatus: it is better to moisten the upper surface of the knife with a

little of the gum. The section is carefully removed with a camel's hair brush and placed in a large vessel of clean water so that the gum may be dissolved out of it, and is then ready for staining. The block is then raised by means of a very slight turn of the large milled head under the apparatus and another section cut.

The mass must not be frozen too hard; if this has been the case the necessary thawing will be hastened by gently breathing on the block. If it begins to thaw a few squeezes of the bellows will bring it to the proper consistency.

## STAINING AND MOUNTING FROZEN SECTIONS.

These processes are best carried out in watch-glasses. No attempt will be made to describe the methods by which frozen sections may be stained for the purposes of bacteriological research, for they are not so suitable as paraffin sections for this purpose. We shall describe the process which would be adopted if a rapid diagnosis were required at an operation or in the post-mortem room. The sections are to be stained in hæmatoxylin (with or without eosin as a counterstain) and mounted in balsam.

The *requisites* are:—Five watch-glasses containing respectively hæmatoxylin, watery solution of eosin (about one per cent.), alcohol (50 per cent.), absolute alcohol, and clove oil; a saucer or other vessel containing water to which a few drops of ammonia have been added; several strips of thin writing paper, each about one inch wide and two inches long; some needles,

which may be mounted in handles; slides, cover-glasses, and balsam.

A section is to be removed from the bowl of water in which it is floating by means of one of the strips of paper; this must be inserted under it, and the section "pinned" in place upon by one of the needles. A special section-lifter may be used, but is not so good. It is then transferred to the watch-glass containing the hæmatoxylin solution, and the staining process is allowed to go on for a minute or two, a fresh section being manipulated whilst it is taking place. The first section is then removed in the same way as before and placed in the water containing the ammonia; it soon turns blue, and when this is the case it is ready to be transferred to the eosin, then into the dilute alcohol, the absolute alcohol (where it should remain for a minute or more) and finally into the oil of cloves. It is then ready to be mounted in balsam. A convenient way in which a section can be transferred to a slide is as follows:—The section is carefully spread out whilst in the oil of cloves, two needles being used for the purpose, and a slip of paper insinuated beneath it. This strip of paper is then drawn slowly out of the liquid, and any folds or creases which may be in the section straightened out with the needles, the excess of the oil of cloves being allowed to drop off whilst this is taking place. The strip of paper is then inverted (the section remaining adherent to the under surface) and placed upon a clean slide and pressed firmly upon it; the pressure squeezes out the greater part of the oil so that the section adheres to the slide and the paper can be stripped cautiously from it. A drop of balsam is then applied, the section covered with a cover-glass, and examined under the microscope.

Where time permits it is a great advantage to rinse the section in distilled or clear rain water after removing it from the hæmatoxylin.

The solution of hæmatoxylin is best bought ready made, as its preparation is somewhat difficult. Delafield's solution is about the best for general work.

A counterstain is not really necessary for diagnostic purposes, and its omission hastens the process somewhat.

### THE PARAFFIN PROCESS.

Tissues which are to be cut in paraffin may be hardened in any of the fluids mentioned above. They are then dehydrated, cleared in chloroform or other fluid which mixes with alcohol and dissolves paraffin, and finally soaked in a mixture of hard and soft paraffin kept just at the melting point. This paraffin should be obtained specially for the purpose; the Cambridge paraffin is the best. It is made in two varieties, the soft, which melts at  $48^{\circ}$  C., and the hard, which melts at  $55^{\circ}$  C. The amounts of each which should be used for embedding depends upon the external temperature; in very hot weather hard paraffin may be used alone, while under average circumstances a mixture of equal parts of each is best.

We shall now proceed to describe the various processes seriatim.

### DEHYDRATION.

This is very simple. The blocks of tissue are placed in weak spirit for a few hours or for a day, then

changed into stronger spirit and so on until absolute alcohol is reached. The slower this process is carried out the better will be the results; in practice the strengths of the successive lots of spirit used may be 40 per cent., 75 per cent., and the strong methylated spirit, and the block may remain in each for twelve hours. Lastly, it goes into two successive lots of absolute alcohol.

In all cases the amount of fluid must be greatly in excess. It is useless merely to cover the block with the spirit.

### CLARIFICATION.

In the next step the alcohol is removed from the tissue and replaced by some fluid which will dissolve paraffin. Fats are dissolved out from the tissues at this stage.

This step is also very simple. The blocks are passed directly from absolute alcohol into chloroform and allowed to remain there until they appear translucent. It is not necessary to use a preliminary bath of a mixture of alcohol and chloroform.

It is a good plan to place the bottle containing the block in a warm place with the cork out for an hour or so before proceeding further, as by so doing the last traces of the alcohol will be removed.

### INFILTRATION WITH PARAFFIN.

This is the stage which presents most difficulties to the home-worker, for it is necessary to keep the block

of tissue soaked in paraffin which is just melted for at least twelve, and more often twenty-four hours. To do this properly involves the use of some sort of an incubator. This might possibly be rigged up out of a tin biscuit-box in the manner already described, though considerably more heat would be necessary, as the paraffin melts at about 50° C. But the writer has often embedded the blocks by placing them in bottles containing the paraffin at such a distance from the fire that the paraffin is never completely melted, but always shows a thin solid layer on the surface. To do this it is only necessary to look at the bottle occasionally and move it a little further from the fire if the paraffin is completely melted and *vice versa*. The process may be stopped at night without any harm resulting, and if the soaking only continues for a few hours at a time it is of no consequence so long as the total period is made up.

### CASTING THE BLOCKS.

Special metal moulds are used in the laboratory (fig. 31). A pill box will do quite well. A small amount of melted paraffin is poured into the box and the piece of tissue is taken from the bottle containing the melted paraffin with a pair of forceps (previously warmed so as to prevent the paraffin from setting upon the points) and placed in the paraffin in the pill box. It is necessary to see that the surface from which sections are to be cut should be placed downwards. The box is then filled up with melted paraffin, and placed in a cool place or surrounded with water. The moment a firm film has formed over the surface the whole is plunged in cold water to hasten the setting of the paraffin; the more

rapidly this takes place the better will the block cut. When the paraffin mass has hardened completely throughout it is trimmed into shape, taking care that the edges of the surface which is to be cut are accurately parallel.

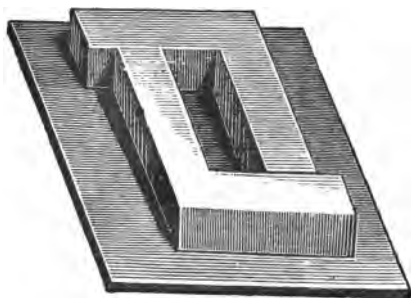


FIG. 32.—L-shaped moulds for embedding in paraffin.

## CUTTING THE SECTIONS.

For cutting sections in paraffin no microtome can be compared with the Cambridge Rocker, but very excellent results can be obtained by the use of the Cathcart microtome already mentioned. The paraffin block containing the piece of tissue is mounted on the freezing plate of the microtome (which must be heated and the lower surface of the block pressed upon it) and the sections cut in the manner described; a very sharp knife is essential and the stroke must be quicker and sharper than is the case when frozen sections are being cut. In another form of the microtome a special inner tube is provided for cutting sections by the paraffin process. The blocks are



retained in place by a clamp and appear in the same position as that occupied by the mass of frozen gum; as the paraffin is not sufficiently hard to be gripped by this clamp they must first be mounted on a piece of wood of a suitable size and shape. This can be cut out of a piece of firewood, and should have one surface left rough; this surface must be dipped in melted paraffin, and the under surface of the block partially melted in the flame and pressed firmly upon it. The piece of wood is then to be placed in the jaws of the clamp and the screw tightened up.

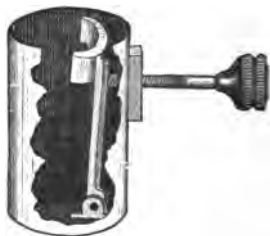


FIG. 33.—Clamp for holding wooden block with the paraffin block.

In the Cambridge Rocker and in some other forms of microtomes the sections adhere to one another at the edges and form long ribbons as they are cut. In the Cathcart microtome this is not the case, and each section must be dealt with separately; it is to be removed carefully from the knife blade with a camel's hair brush or a finely pointed pair of forceps and placed upon the surface of a bowl of water just hot enough to warm the paraffin without melting it. When this is done the sections will spread out and lose all the creases, and are then ready to be mounted on slides or cover-glasses.

It often happens that the sections roll up on the knife. In this case they must be placed on the surface of *cold* water and an attempt made to straighten them out by careful brushing with a camel's hair brush; when fairly flat they are to be lifted up on a slide or piece of paper (dipped into the water and insinuated below them) and transferred to the hot water as before. But the rolling of the sections may often be prevented by sharpening the knife, by re-imbedding the tissues in harder or in softer paraffin according to the weather, or by varying the angle which the knife edge makes with the glass runners of the microtome. These devices can only be learnt by experience.

When the sections are flattened out on the surface of the hot water they are ready to be mounted upon slides or cover-glasses; slides are by far the best for beginners. The slides (or cover-glasses) must be perfectly clean, and are best kept in methylated spirit until they are to be used, and the spirit not wiped off. Each slide is then inserted separately into the water in an oblique position and the section moved until it lies over the centre; the slide is then raised out of the water and carries the section out with it.

The excess of water is now to be removed by a piece of blotting or filter paper, and the slide placed in the warm incubator for a few hours. At the end of this time the sections will adhere by atmospheric pressure (like a boy's leather sucker to a stone) and will not come off in the subsequent processes. If an incubator is not at hand the slides may be placed near the fire (protected from dust) and kept at the body temperature or a little higher for a few hours; the exact temperature does not matter, and no harm will result if the paraffin melts.

In the older methods of fixing sections to the slides various forms of cements had to be used, and were a great disadvantage. They are quite unnecessary except for sections of the central nervous system; if these are being dealt with the slide must be coated with a very thin layer of a solution of egg-albumen in water before the section is laid upon it. The process is then exactly the same as before.

## STAINING AND MOUNTING PARAFFIN SECTIONS.

We will suppose that the sections have been cut, flattened out on hot water, and caused to adhere to slides, and shall describe in general terms the steps through which they must be taken before they are ready for examination. In the first place, it is obvious that the paraffin which permeates all parts of the section and surrounds it on all sides must be removed; and this is done by pouring xylol, benzine, or turpentine upon it. At least two supplies of the fluid should be used, and it should be allowed to act for at least two minutes, the slide being rocked all the time. We have now removed the paraffin, and the next step is to remove the xylol or other solvent; this is done by means of absolute alcohol. At least two lots should be used, and it should be allowed to act for two minutes. The slide is then washed in water, and is ready for staining. When the section is wet with xylol it will be quite transparent; this is because the refractive index of the xylol is almost the same as that of glass and the rays of light which come through the section are not bent. But when the

alcohol is added the section will suddenly become opaque, and for the opposite reason.

If there is a milkiness on the section or slide when the water is poured on it is a sign that the xylol has not been completely removed; xylol will not mix with water and forms an emulsion with it. If this should happen you must give the section another dose of absolute alcohol.

It is an advantage to wipe the surface of the slide (of course avoiding the section) before going from one fluid to another.

A cardinal rule in dealing with paraffin sections is *never to let the section get dry* from the moment the first dose of xylol is added until the final mounting in balsam.

The methods of staining which are in use are legion, and it would be far beyond the scope of this book to describe even a few of those which are used in histological work and to give indications for their use. It will be sufficient to describe (1) a method suitable for the diagnosis of tumours, &c., and for ordinary histological purposes, (2) a method of staining to demonstrate bacteria which stain by Gram's method, (3) a method for bacteria which do not stain by Gram's method, and (4) the process for demonstrating tubercle bacilli in the tissues.

(1). Staining sections for histological purposes:—

1. Xylol, two lots (to remove paraffin).
2. Absolute alcohol, two lots (to remove xylol).
3. Water (to remove the alcohol).
4. Stain with hæmatin (or hæmatoxylin) for ten minutes or more according to the nature of the specimen and the condition of the stain. The exact length of time can only be learnt by trial, but ten minutes will be about right. Rinse in distilled water.

5. Wash thoroughly in tap water, continuing the washing until the sections have a decidedly blue tinge. The hæmatoxylin compounds are very much like litmus, being red in presence of acids and blue in presence of alkalis; the sections are to be coloured blue, and the necessary alkali is contained in the tap-water. It will hasten the process to rinse them in a very dilute solution of ammonia.

6. Stain in watery eosin for a minute or so. This is the *counterstain*. The hæmatin will stain all nuclei blue, but will scarcely tinge anything else; the eosin is added to stain other structures a pale pink and thus make them more visible. It stains almost instantaneously.

7. Wash off the eosin under the tap.

The sections are now stained. But they are opaque and not in a suitable condition to be examined under the microscope, and are to be rendered transparent by being mounted in balsam. Now this cannot be done in the same way as was used in the mounting of films, for the drying would cause the sections to shrivel and obscure their structure. The water is to be removed, it is true, but by the use of absolute alcohol; at least two lots should be used, and the slide rocked from time to time. Then the alcohol (which will not mix with balsam) is to be removed by the use of xylol, balsam added, and the section covered with a cover-glass. The remaining steps are therefore :—

8. Absolute alcohol, two lots (to *dehydrate*).

9. Xylol, two lots (to render the section permeable to balsam).

10. Balsam and a cover-glass.

The last three steps are practically the same as the first three, but in the reversed order, and similar phenomena are seen. The section is opaque whilst wetted

with the alcohol and becomes transparent when the xylol is added; and this transparency is the proof that the steps have been carried out properly. If the section looks opaque when held against a perfectly dark background an additional dose of alcohol must be used, and the xylol applied again.

Hæmatoxylin may be used in exactly the same way as hæmatin in the above process; it stains more quickly, but does not give quite such beautiful results.

(2). Gram's method as applied to sections; suitable for sections of diphtheritic membrane, organs containing anthrax bacilli, streptococci, staphylococci, &c.

1. Xylol, two lots.
2. Absolute alcohol, two lots.
3. Water. These steps are always the same with paraffin sections, no matter what stains are to be used subsequently.
4. Aniline gentian violet—five minutes.
5. Gram's iodine solution—three minutes or more.
6. Absolute alcohol or methylated spirit—*until no more colour comes out*. This step is best carried out as follows:—Hold the slide by one end, keeping the fingers clean by using a duster or pair of dissecting forceps, and pour a little spirit on the section; rock it gently from side to side and notice the clouds of colour which it takes up. After a little time pour off the spirit and add a fresh lot; repeat the rocking, and pour off again. Do this until the spirit comes away quite clean and does not take up any colour from the section. This may take a long or a short time, and no definite rules can be laid down.

In some cases decolorisation can be carried out best by the use of clove oil. This is applied when the spirit is wet with absolute alcohol (for it will not mix with

water) and must be entirely removed with the same fluid before the section is mounted, or it will cause it to fade. Clove oil is a very powerful decolorising agent, and requires careful use, or the colour may be removed from the bacteria.

7. Eosin—half a minute or more. This is a counter-stain, and is used to demonstrate the structural elements, which are not coloured by the gentian violet. It may be omitted in some cases.

8. Absolute alcohol—two lots. To remove the water.

9. Xylol—two lots, or until the section becomes transparent.

10. Balsam and a cover-glass.

This method of staining is very easy of application, and the results are exceedingly beautiful; bacteria which take the stain are coloured blue or violet, and actively-dividing nuclei and keratin are stained in the same way, while all other structures are stained pink.

(3). Method for bacteria which do not stain by Gram's method; suitable for sections of typhoid ulcers, lymphatic glands containing plague bacilli, &c.

The problem before us in this case is not at all easy of solution. In the first place, the stains which colour the bacteria also colour the tissues, especially the cell-nuclei; the bacteria are easy to stain, but it is difficult to stain a section in which there is good differentiation. In the second place, the stains which are used for bacteria are all soluble in alcohol; but alcohol is used to dehydrate the sections. The following method will be found to serve fairly well in most cases, though it requires a certain amount of practice for its successful accomplishment.

1, 2, and 3. Xylol, alcohol, and water, as before.

4. Stain in carbol-thionin for ten minutes or quarter of an hour.

5. Wash in running water for ten minutes or longer. This removes the stain from the tissues before decolorising the bacteria, and a fairly differentiated specimen may be obtained if the processes of staining and washing are carried out for suitable lengths of time.

Unna's polychrome methylene blue may be used in a similar manner, and gives even better results. The staining should be continued for about ten minutes and decolorisation effected by *very short* immersion in dilute acetic acid (about  $\frac{1}{2}$  per cent.) followed by a good washing in pure water.

6. Remove as much water from the section as you can without actually drying it by the cautious use of clean blotting paper. Then apply anilin oil until the section becomes perfectly translucent. Anilin oil mixes with water on the one hand and xylol on the other and can be used for dehydration just as alcohol was; the process is slower and several lots of the oil must be used.

7. Wash off all the anilin oil by successive applications of xylol. The permanence of the preparation will depend on the thoroughness with which this step is carried out.

8. Balsam and cover-glass.

(4). Staining sections to demonstrate the tubercle bacillus. Applicable to the leprosy bacillus also.

1, 2, and 3. Xylol, alcohol, and water, as before.

4. Carbol-fuchsin heated until the steam rises for five minutes or longer, care being taken that the section does not dry up. Or the slide may be immersed in the stain and kept in a warm place for twenty-four hours.

5. Dilute sulphuric acid until only a faint pink tinge appears after washing. This will generally require an immersion of ten minutes or more.



6. Methylene blue for three or four minutes. Some of the stain comes out in the alcohol, so that the section must be stained more deeply than will be required ultimately.

8. Rinse off the blue stain in water, and then remove the greater part of the latter with blotting-paper; this is to render the dehydration more rapid.

9. Absolute alcohol—two lots in rapid succession.

10. Xylol.

11. Balsam and cover-glass.

## APPENDIX.

## I.

The following tables may be useful if stains, &c., have to be prepared when the metric weights and measures are not at hand. The equivalents given are not absolutely accurate, but are sufficiently near for most purposes.

1 Litre . . .	35½ fluid ounces.
100 C.C. . . .	3½ fluid ounces.
1 Gramme . . .	15·43 grains.
1 Fluid ounce . .	28·4 c.c.
1 Fluid drachm . .	3·5 c.c.
1 Grain . . . .	·064 gramme.

To convert grammes per litre into grains per ounce multiply by the factor  $\frac{7}{128}$ . Thus 10 grammes per litre =  $10 \times \frac{7}{128} = 4·4$  grains per ounce.

To convert cubic centimetres per litre into minims per ounce multiply by the factor  $\frac{1}{2}$ . Thus 25 c.c. per litre =  $25 \times \frac{1}{2} = 12$  minims per ounce. (A close approximation is given by multiplying by  $\frac{1}{2}$ .)

To convert degrees Centigrade into degrees Fahrenheit multiply by the factor  $\frac{9}{5}$  and add 32.

## II.

## NEISSER'S METHOD OF STAINING THE DIPHTHERIA BACILLUS.

The following method has been placed in the Appendix as there is some doubt as to its value. It is a

method by which the so-called polar bodies are stained with methylene blue, while the rest of the organism is coloured a faint yellowish-brown with Bismarck brown.

Cultures should be made on blood-serum and should not be less than nine nor more than twenty-four hours old. Films are spread in the ordinary way and stained for half a minute in

Methylene blue . . .	1 gramme.
Alcohol (96 %) . . .	20 c.c.
Glacial acetic acid . . .	50 c.c.
Water . . . . .	950 c.c.

They are then washed and treated for half a minute with

Bismarck brown . . .	5 grammes.
Water . . . . .	1000 c.c.

The polar bodies are small spheres which are contained in the bacilli, there being usually two in each bacillus, one at each end. In a film specimen of the true diphtheria bacillus stained in this way they appear as very minute dark blue or black dots, which may easily be mistaken for cocci; the bodies of the bacilli are often almost invisible. According to some authorities the presence of these granules in young cultures of bacilli which present the morphological characters of the diphtheria bacillus, is proof of their virulence, whilst their absence proves the cultures to be of the non-virulent "pseudo-diphtheria" bacillus. The method can also be applied to films made directly from the swabs, and recent researches seem to prove that the results thus obtained are of considerable diagnostic value.

If this method be adopted the films must be very

carefully searched, as it often happens that characteristically stained bacilli may be seen in one part of the field, while they are entirely absent elsewhere. This is especially true of films made direct from the swabs.

The author is inclined to attach very considerable importance to a positive result obtained with this method of staining, but would not consider a negative result as indicating the absence of the diphtheria bacillus.



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